A CHEMOENZYMATIC APPROACH TO MORPHINE AND OTHER OXYGENATED ALKALOIDS. TOTAL SYNTHESIS OF NARCICLASINE

By

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Para mi querida Alejandra, quien me dio a Joaquin.
(To my beloved Alejandra, who gave me Joaquin)

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TABLE OF CONTENTS

page

ACKNOWLEDGMENTS iii

LIST OF TABLES vi

LIST OF FIGURES vii

ABSTRACT xi

1 INTRODUCTION 1

2 HISTORICAL BACKGROUND 4

2.1. Narciclasine 4
2.2. Morphine 29

3 RESULTS AND DISCUSSION 50

3.1. Isolation and Identification of New Metabolites 50
3.2. Chemoenzymatic Synthesis of Unnatural Amino Acids. Approach to Morphine Synthesis 73
3.3. Chemoenzymatic Synthesis of Narciclasine 93

4 CONCLUSION 106

5 EXPERIMENTAL DETAILS 111

5.1. General Procedures 111
5.2. New Metabolites 112
5.3. Unnatural Amino Acids 114
5.4. Synthesis of Narciclasine 129
5.5. Miscellaneous Compounds 138

REFERENCES 142

APPENDIX: SELECTED SPECTRA 151

BIOGRAPHICAL SKETCH 228

LIST OF TABLES

Table		page
1.	Historical data for some Amaryllidaceae alkaloids.	5
2.	^{1}H-NMR data of natural narciclasine recorded at 500 MHz by Evidente	11
3.	^{13}C-NMR data of natural narciclasine recorded at 69 MHz by Evidente.	12
4.	Influence of the addition of cosolvents and Lewis acids in the Ireland-Claisen rearrangement of amino esters	34
5.	Kazmaier results in the chelated-enolate Claisen rearrangement using various salts as chelating agents.	36
6.	Summarized results for cyclic substrates in the chelated-enolate Claisen rearrangement	37
7.	Characteristic chemical shifts of *R*- and *S*-boronic esters of microbial metabolites.	58
8.	Results obtained in the biotransformation of various oxygenated biphenyls.	65
10.	Results obtained in the epimerization experiments.	90

LIST OF FIGURES

Figure page

1. Examples of dioxygenases metabolites utilized in natural product synthesis. 2

2. A disconnection of the morphine and narcissus alkaloid skeletons.....3

3. Linear relationship between the per cent inhibition of growth of the lateral radicles of wheat and the log of the concentration of narciclasine.....7

4. First proposed structure of narciclasine (**8**) and structure of narciclasine degradation product: cotornic acid (**9**). 8

6. X-ray structure of narciclasine tetraacetate.....10

7. NMR shifts assignment and coupling constants of narciclasine.....12

8. Biosynthesis of narciclasine from *O*-methyl-norbelladine.....13

9. Inference of narciclasine absolute stereochemistry from biosynthetic studies. 14

10. Different options for the final closure of the B ring.....16

11. Simple disconnection of the dioxygenated nucleus in 7-*deoxy*-phenathridone alkaloids compared to their trioxygenated counterparts. 17

12. Heathcock transamination approach to pancratistatin analogs. 18

13. Hudlicky's approach to pancratistatin from an optically pure aziridine. 18

14. Problems observed in the closure of B ring by Hudlicky *et al.* 19

15. Pancratistatin ring B closure via amidation in aqueous media. 20

16. Hudlicky's disconnection of lycoricidine. 22

17. Preparation of the C Ring and Diels-Alder partner for the synthesis of lycoricidine.....22

18. Coupling of the A and C rings of lycoricidine via nitroso Diels-Alder. 24

19. Preparation of the aminoalcohol precursor of lycoricidine.....24

20. Final steps in Hudlicky's synthesis of lycoricidine. 24

21. Asymmetric branch of Rigby's synthesis of narciclasine. 25

22. Preparation of the aromatic fragment of narciclasine. 26

23. Coupling of the aromatic and asymmetric fragments. 27

24. Closing of ring C ring via eletrocyclic reaction. 28

25. Elaboration of the asymmetric portion of narciclasine and final deprotection. 28

26. Examples of naturally occurring non-proteinogenic amino acids. 29

27. Examples of unnatural amino acids. 30

28. Synthesis of amino acids via oxazole rearrangement. 31

29. Four possible pathways of reaction in a enolate Claisen rearrangement. 32

30. Bartlett comparison between the oxazole and enolate methods for identical substrates. 33

31. Kazmaier chelated-enolate Claisen rearrangement. 35

32. Asymmetric version of the chelated-enolate Claisen rearrangement. 38

33. First steps in the Rapoport synthesis. Stereospecific preparation of a rearrangement precursor. 41

34. Orthoester Claisen rearrangement and elaboration to an Evans intermediate. 42

35. Parsons' original approach to the morphine skeleton. 43

36. Parsons' approach to morphinan **111** via 2,3-dipolar cycloaddition 44

37. Parsons' final steps in the synthesis of morphine. 46

38. Mulzer's preparation of an advanced intermediate via Eschenmoser-Claisen rearrangement. 47

39. Formation of the E ring and final elaboration to dihydrocodone (**125**). 48

40. 1-Bromo-2,3-difluorobenzene and its two possible metabolites. 53

41. Chemical proof of the regiochemistry of substituted benzene **126**. 55

42. Structures of the four possible diasteromeric boronic esters that could have been found. Only compounds **133** and **134** were detected. 56

43. Formation of bis-phenols by biphenyl dioxygenase dihydroxylation followed by acid-catalyzed aromatization. 59

44. Oxidation of synthetically relevant biphenyls with toluene dioxygenase. 60

45. Preparation of substrate **141**. 61

46. Preparation of trioxygenated biphenyl **142**. 64

47. Strategy for confirming the stereochemistry of the new metabolites. 66

48. Preparation of the optically pure organostannane derivative **159** from iododiol **153**. 68

49. Preparation of diols **154** and **155** by two independent routes. 69

50. Biooxidation of *meta*-disubstituted benzenes. 70

51. Preparation of a conduramine derivative from diol **43** to confirm its absolute stereochemistry. 72

52. A disconnection or morphine that sets the stereochemistry of C-9 and C-14 at an early stage of the synthesis. 73

53. Preparation of enantiomerically pure 2,3-dimethoxybiphenyl diol by Suzuki coupling. 77

54. Preparation of amino esters precursors for the chelate-enolate Claisen rearrangement. 79

54. Chelated-enolate Claisen rearrangement of biphenyl amino ester **194**. 80

55. Lactonization of amino acid **197**. 82

57. Crystal structure of lactone **203**. 85

58. Summary of the results in the rearrangement of amino esters **192 - 196**. 86

59. Two possible transition states in the rearrangement of amino esters **192-196**. 88

60. A non-chelated boat-like transition state could dictate the stereochemistry of the products. 89

61. Literature precedence and preliminary results on the C10-C11 closure. 92

62. Convergent strategy toward narciclasine from two aromatic building blocs. 93

63. Generation of the "biphenyl diol" precursor. 94

64. Manipulation of diol **167** to allow the efficient coupling with borate **236**. 95

66. Cleaving of the NO bridge in oxazine **171**. 97

67. Suzuki coupling. 99

68. Three operations synthesis of ketone **241** from a non-chiral starting material. 100

69. Setting of the stereocenters in narciclasine. 101

70. Final closure and deprotection. 103

71. Summary of narciclasine synthesis. 105

72. Two previous examples of custom biotransformations projected for use in synthesis. 107

73. Alternative approach to morphine alkaloid. 108

74. A possible approach to the enantiomers of lycoricidine and narciclasine. 110

Abstract of Dissertation Presented to the Graduate School
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Chairman: Tomas Hudlicky
Major Department: Chemistry

Four new metabolites of toluene dioxygenase degradation of substituted aromatics were isolated: 3-bromo-4,5-difluoro-3,5-cyclohexene-1,2-diol, 3,5-dibromo-3,5-cyclohexene-1,2-diol, 3-(2-methoxyphenyl)-3,5-cyclohexene-1,2-diol, and 3-(2,3-dimethoxyphenyl)-3,5-cyclohexene-1,2-diol. All metabolites were characterized and their absolute configuration confirmed by synthesis or spectrometric methods.

The new compounds were evaluated as practical synthons for approaches to oxygenated alkaloids and unnatural amino acids. The biphenyl derivatives were utilized in an approach to the morphine skeleton via a chelated-enolate Claisen rearrangement. The dibrominated metabolite was used as starting material in a short total synthesis of narciclasine.

CHAPTER 1
INTRODUCTION

Cis-cyclohexadienediols obtained by enzymatic dihydroxylation of arenes have been used in organic synthesis since the early eighties. The first example of a natural product synthesized from a microbial diol was the racemic synthesis of pinitol by Ley *et al.*[1] Several groups around the world rapidly followed his disclosure, and the exponentially increasing amount of work produced has been reviewed in several instances.[2-7] In spite of the fact that more than 200 examples of asymmetric diols have been isolated or detected since the elucidation of the metabolic degradation pathway of aromatics in prokaryotes by Gibson *et al.*,[8] the great majority of the applications in synthesis are limited to a handful of such metabolites (Figure 1). This is explained in part by the low yields in which many of the metabolites were originally isolated, but also by some reluctance from the traditional organic community to incorporate tools from external fields. Such factors played a role in the delay. Currently, the development of a powerful constitutive mutant strain of *Pseudomonas* (*P. putida* UV4)[9] as well as several recombinant strains of *E. coli* which express dioxygenases[10-12] allow the oxidation in good yields of aromatic substrates structurally very different from the natural substrates of the enzymes.

Several alkaloids as well as other natural products contain aromatic rings linked to structures containing asymmetric centers. Such architectures present attractive targets for

a strategy that makes use of the underutilized metabolites: biphenyl- and *meta*-substituted benzene-diols as starting materials, Figure 2.[13] For example, a disconnection of morphine (**1**) can be envisioned where the link between the A and C rings is already present in biphenyl diol **6**. An approach to morphine that utilizes this starting material and that also sets the stereochemistry at C9 and C14 (morphine numbering) is presented in this dissertation.[14]

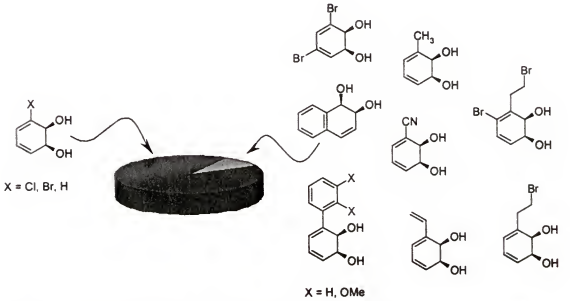


Figure 1. Examples of dioxygenases metabolites utilized in natural product synthesis.

In the case of the Amaryllidaceae alkaloid narciclasine (**2**) the disconnection shown in Figure 2 required biphenyl diol **7**, whose regiochemistry is unattainable by means of direct toluene dioxygenase oxidation of the corresponding biphenyl. In the synthesis of narciclasine,[15] also presented in this dissertation, this problem is bypassed by the realization that a halogenated metabolite of type **8**, provides a pattern of substitution of the type present in **7**.

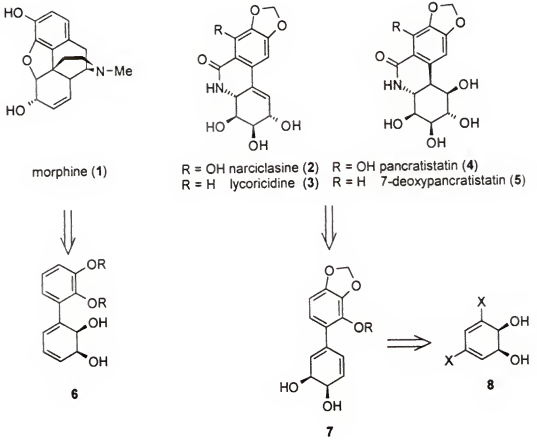


Figure 2. A disconnection of the morphine and narcissus alkaloid skeletons.

CHAPTER 2
HISTORICAL BACKGROUND

The historical background of this work includes a review of the biological and chemical information about narciclasine as well as a discussion of the previous published preparation of narciclasine by Rigby. The preparation of amino acids via Claisen rearrangement are reviewed, with emphasis in the chelate-enolate Claisen rearrangement developed by Kazmaier. Finally, three preparations of morphine that include a Claisen rearrangement as a key step are described and discussed.

2.1. Narciclasine

Extracts from *Narcissus poeticus* L. (daffodil) have been used in traditional medicine for a very long time.[16] However, only in the second half of the twentieth century some of the alkaloids isolated from this family have gained serious attention because of their relevant biological activity. Some of the main alkaloids isolated from species of the Amaryllidaceae family together with their abundance and reported biological activity are shown in Table 1.

The intriguing structures, superb biological activity and natural scarcity of these molecules have triggered the interest of the synthetic community. Although, these efforts have resulted in numerous approaches and a few total syntheses, none of the approaches delivers the necessary amounts of material for medicinal use.

Table 1. Historical data for some Amaryllidaceae alkaloids.

alkaloid	abundance (mg/Kg of dry weight)	biological activity	asymmetric synthesis[17,18]
Lycoricidine (**3**)	4-10	antineoplastic[19]	Hudlicky, 1992
Narciclasine (**2**)	18-200	antineoplastic[19] antimitotic[20]	Rigby, 1997[21]
7-*deoxy*-pancratistatin (**5**)	7-10	antineoplastic	Hudlicky, 1995[22]
Pancratistatin (**4**)	4-25 (arizona) 150 (Hawaii)[23,24]	antineoplastic[24,25] antiviral	Hudlicky, 1995[26,27]

2.1.1. Isolation and Biological Activity

Narciclasine is found in *Lycoris radiate*[19] as well as several daffodil species (*Narcissus tazetta* L., *N. incomparabilis* Mill. var. "Helios" and "Sempre Avanti", *N. pseudonarcissus* L. var. "King alfred", and *N. triandrus* L. var. "Thalia" and "Tresamble".[28] The abundance of the alkaloid varies with the species yielding between 18 to 200 mg/Kg of wet plant material. The extraction is performed by maceration of the bulbs in 95% ethanol for 24 hours followed by repeated filtration, evaporation, extraction, chromatography and finally crystallization.[28]

An improved and easier method of isolation has been reported by Evidente.[29] The fresh bulbs were dried at 40°C for three days, then milled, and extracted three times with 0.1 N NaOH by overnight maceration. After acidification (pH=2.0) and extraction with ethyl acetate the residue was purified by column chromatography and finally

recrystallized from acetic acid. This procedure afforded narciclasine as white needles in a yield of 18-55 mg/kg of fresh bulbs depending on the species.

Narciclasine was detected because crude extracts of *Narcissus* bulbs were shown to inhibit the growth of the radicles of wheat grains at concentrations as low as 5 μg/ml.[20] The pure alkaloid showed strong inhibition activity at concentrations below 1 μg/ml, Figure 3.

Narciclasine has been also assayed for its action against sarcoma 180 in ascites form and its bacteriostatic effect has been determined to be evident at 8 μg/ml. The LD_{50} in mice has been measured and found to be 5 mg/kg by subcutaneous injection.

2.1.2. Structure Elucidation

In 1968, shortly after the first isolation of narciclasine by Ceriotti,[20] structure **8** was proposed based on degradation studies and spectroscopic data (Figure 4).[28] The presence of a phenolic hydroxyl group was suggested by the solubility in alkalis, IR spectrum and batochromic effect observed in the UV spectra. The infrared spectrum also indicated the presence of hydroxyl, carbonyl and methylenedioxy groups. The methylenedioxy moietywas further confirmed by the evolution of formaldehyde when narciclasine was subjected to sulfuric acid treatment. The presence of a double bond was determined by catalytic hydrogenation and the UV spectra of the product suggested that the olefin must be in conjugation with the aromatic ring.

Zinc distillation of narciclasine produced phenanthridine. This fact suggested that the backbone of the alkaloid must contain at least three rings. Methylation with diazomethane followed by permanganate oxidation yielded cotornic acid (**9**), as a further confirmation of the structure of the aromatic portion. Unfortunately, with all the

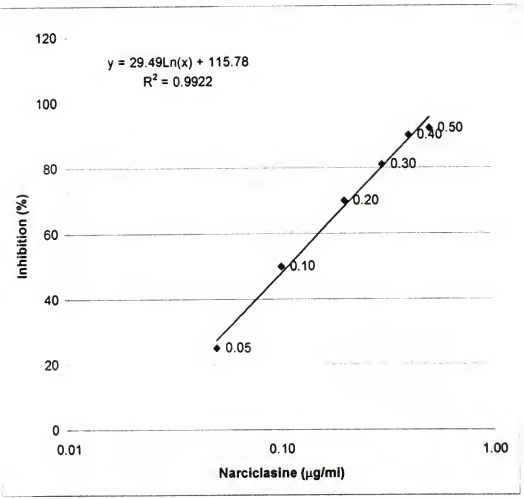
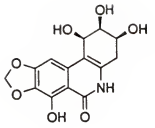


Figure 3. Linear relationship between the per cent inhibition of growth of the lateral radicles of wheat and the log of the concentration of narciclasine (adapted from published work by G. Ceriotti).[20]



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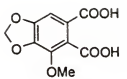

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Figure 4. First proposed structure of narciclasine (**8**) and structure of narciclasine degradation product: cotornic acid (**9**) as reported by Piozzi *et al.*[28]

functional groups identified and the basic carbon skeleton determined, the authors were not able to suggest the correct structure. This is even more striking, after observing the proton NMR spectrum published in the paper (Figure 5). Although at 100 Mhz the signals assigned to the methylenedioxy group (6.12 ppm) and to proton "H-1" (6.18 ppm) are not resolved, it seems adventurous to place such allylic proton so far downfield. The failure to recognize the signal at 6.18 ppm as a vinylic proton induced Piozzi *et al.* to propose the incorrect structure **8**.

Later Immirzi and Fuganti[30] published the X-ray structure of narciclasine tretraacetate, prepared from the natural alkaloid, and corrected the missassigment (Figure 6). To the best of our knowledge, an X-ray analysis of the free alkaloid has been not been reported to date.

Although narciclasine was isolated in 1968, no detailed NMR data was published on the pure alkaloid until 1989 when the first ^{13}C-NMR carbon spectra was reported[31] and 1991 when a report by Evidente[29] discussed the full ^{1}H- and ^{13}C-NMR spectra. A listing of the ^{1}H-NMR chemical shifts and the current assignment is shown in Table 2.

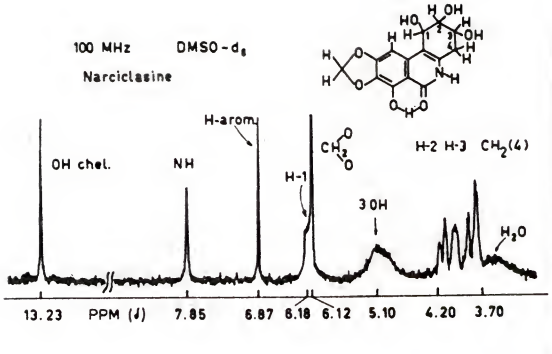


Figure 5. First published spectrum of narciclasine (adapted from published work by Piozzi *et al.*).[28]

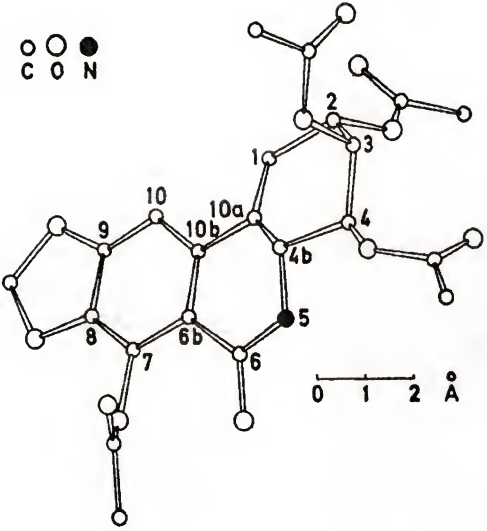


Figure 6. X-ray structure of narciclasine tetraacetate (adapted from a report by Immirzi *et al.*).[30]

The assignments were made by performing a series of decoupling experiments. No two dimensional experiments on narciclasine were reported in this work. The ^{13}C-NMR data (without assignment of multiplicities) as reported by Evidente is shown in Table 3. A complete assignment of the NMR shifts and coupling constants based on Evidente's work is shown in Figure 7. Note that the signals corresponding to the hydroxyls on C-2, C-3, and C-4 are not reported in this paper. This fact suggests that the deuterium exchange is faster for these groups than for the phenolic alcohol. This conclusion is in agreement with the tight intramolecular hydrogen bond between the phenolic proton and amidic carboxyl indicated by the chemical shifts.

Table 2. ^{1}H-NMR data of natural narciclasine recorded at 500 MHz by Evidente.[29]

proton	CD_3SOCD_3	CD_3OD
H-1	6.15(ddd, J=4.9, 2.4, 1.0)	6.17(ddd, J=4.9, 1.2, 2.4)
H-2	4.01(ddd, J=4.9, 2.4, 1.2)	4.23(ddd, J=4.9, 2.4, 1.2)
H-3	3.69(ddd, J=2.4, 2.4, 1.0)	3.92(ddd, J=2.4, 2.4, 1.2)
H-4	3.78(dd, J=8.5, 2.4)	3.90(dd, J=9.6, 2.4)
H-4a	4.18(ddd, J=8.5, 2.4, 1.2)	4.35(ddd, J=9.6, 2.4, 1.2)
H-10	6.84(s)	6.75(s)
H-11a	6.09(d, J=1.2)	6.02(d, J=1.2)
H-11b	6.08(d)	6.00(d)
HN-5	7.88(s)	-
HO-7	13.24(s)	-

Table 3. ^{13}C-NMR data of natural narciclasine recorded at 69 MHz by Evidente.[29]

carbon	shift	carbon	shift
C-1	124.7	C-7	168.9
C-2	69.1	C-8	152.3
C-3	72.3	C-9	144.8
C-4	68.8	C-10	95.7
C-4a	52.8	C-10a	132.1
C-6	172.1	C-10b	133.3
C-6a	129.2	C-11	102.0

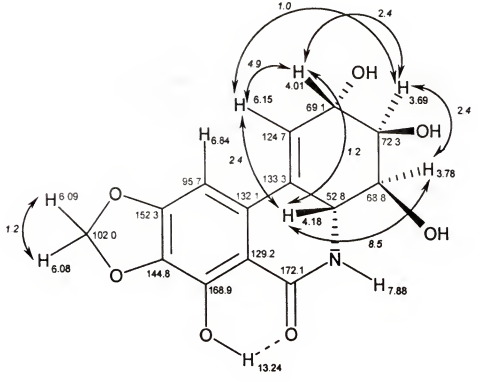


Figure 7. NMR shifts assignment and coupling constants of narciclasine, according to Evidente[29] (^{1}H and ^{13}C shifts are in ppm relative to d^6-DMSO; coupling constants are in Hertz).

2.1.3. Biosynthesis

The biosynthetic pathway of narciclasine has been studied by Fuganti *et al.*[32-34] Feeding experiments using single and double-labeled precursors first proved that narciclasine can be biosynthesized from *O*-methyl-norbelladine (**10**) (Figure 8).[32] A *para-para* coupling between the two aromatic rings in **10** was proposed to establish the connection amid C10a-C10b. [3',5'-$^{3}H_2$; *O*-methyl-^{14}C] *O*-methyl-norbelladine was prepared and fed to two different species of *Narcissus*, that incorporated the labels into narciclasine suggesting the pathway shown in Figure 8.

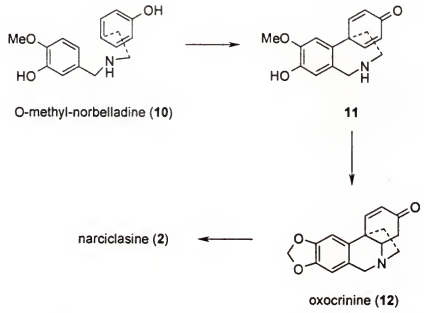


Figure 8. Biosynthesis of narciclasine from *O*-methyl-norbelladine.

Important structural information was inferred from biosynthetic studies. Fuganti and Mazza[34] proposed the correct absolute configuration of narciclasine before the crystal structure of the tretraacetate was reported.[30] They proved by feeding experiments using

optically active compounds that narciclasine was derived from vittatine (**13**) and not from its enantiomer crinine (**14**) (Figure 2). The optically active precursors were obtained by biotransformation of [3',5'-$^{3}H_2$; *O*-methyl-^{14}C] *O*-methyl-norbelladine into the former compounds using *Pancratium maritimun*in and *Nerine bowdenii* respectively. "Texas" and "Twink" daffodil did not incorporate optically active crinine, while they did incorporate labeled samples of (+/-)-crinine and optically pure vittatine. Since the absolute stereochemistry of vittatine is known, and its configuration at C-4a is not likely to change in the biosynthetic steps to follow, it could be inferred that the absolute stereochemistry of narciclasine was as shown (Figure 9).

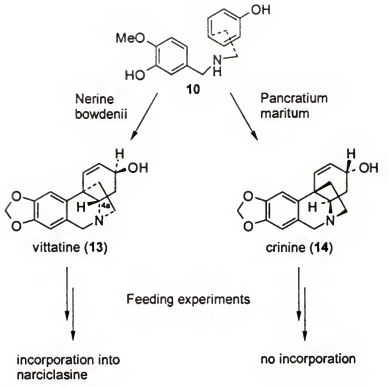


Figure 9. Inference of narciclasine absolute stereochemistry from biosynthetic studies.

2.1.4. Synthesis

All published approaches to phenanthridone alkaloids present some common motives. They are all convergent strategies in which a polyoxygenated aromatic ring is connected to a functionalized cyclohexane. After the two substructures are linked and all the stereocenters are set correctly, the B ring is closed by establishing a second connection between rings A and C, Figure 1. Because of the rich functionality present in ring C, the options for this final key step are quite diverse. In several approaches to lycoricidine (**3**) a disconnection of type I was utilized.[35-38]

Although these approaches proved to be powerful (the nine steps synthesis of **3** by Hudlicky is the shortest preparation to date)[35] no second generation synthesis that applies a type I disconnection has appeared in the literature. A possible explanation for this fact could be the diverse yield reported for the Heck closure in this system (Chida: 68%, Hudlicky: 27%, Martin: 51%) indicating that the exact conditions for the reaction are hard to reproduce. Another, explanation, relies in the arduous preparation of the trioxygenated piperonal derivative of type **17** that could expand the methodology toward narciclasine (**2**) and pancratistatin (**4**), Figure 2. Finally, the results reported by Pettit[39] who attempted to prepare pancratistatin from narciclasine suggested that a route toward **4** via dihydroxylation of the double bond of narciclasine (**2**) would be laborious. This issue, though not thoroughly investigated, might have made the Heck approach less appealing.

2.1.4.1. Hudlicky's synthesis of pancratistatin.

A disconnection of the amidic bond (type II, Figure 10) has infrequently been used for the final closure. Only Hudlicky's first generation synthesis of pancratistatin[22,26]

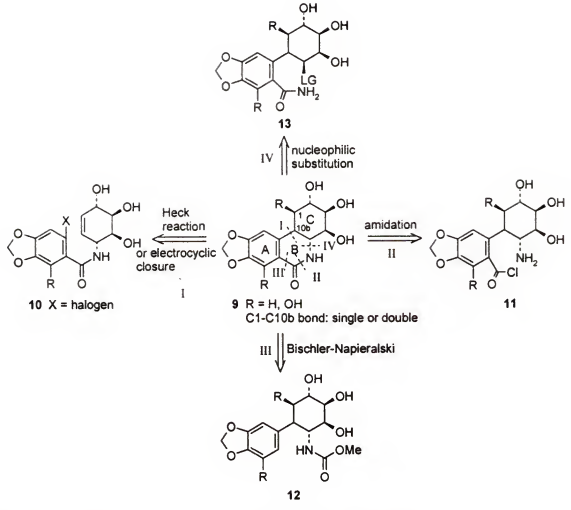


Figure 10. Different options for the final closure of the B ring.

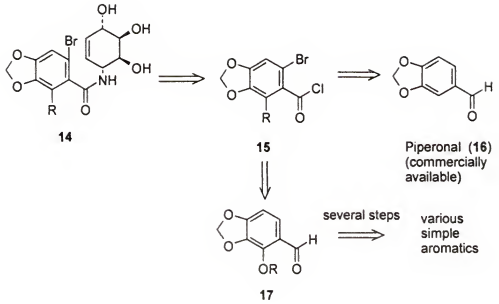
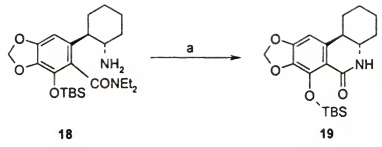


Figure 11. Simple disconnection of the dioxygenated nucleus in 7-*deoxy*-phenathridone alkaloids compared to their trioxygenated counterparts.

utilized this strategy and the formation of the challenging *trans*-lactam ring only succeeded after careful optimization of the reaction conditions. The methodology followed by Hudlicky and Tian in their synthesis was inspired by a model study by Heathcock.[40] He had reported a transamination approach to *deoxy*-analogs of type **19** by means of *s*-BuLi at –15 °C (Figure 12).

This transformation seemed amenable for application in the aziridine opening-based strategy followed by Hudlicky (Figure 13). Unfortunately, the scope of the reaction turns out to be very limited since it did not tolerate the presence of a double bond on C1-C2 (pancratistatin numbering). Apparently, the olefinic bond present in intermediate **20** increased the acidity of C10b leading to epimerization or elimination.



Conditions: (a) *s*-BuLi, THF, -15°C, 56%

Figure 12. Heathcock transamination approach to pancratistatin analogs.

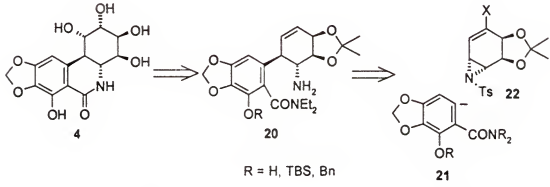
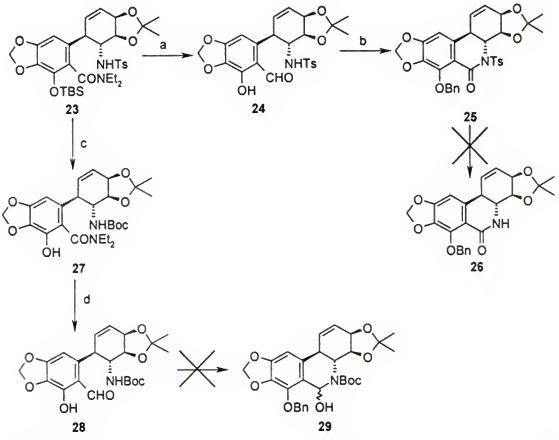


Figure 13. Hudlicky's approach to pancratistatin from an optically pure aziridine.

With these results in hand Hudlicky and Tian abandoned transamination and transformed the formamide **20** into aldehyde **24** via tosylated amine **23** (Figure 14). This material did undergo the ring closure but disappointingly, the resulting phenanthridone **25** resisted all attempts of detosylation, including sodium amalgam, sodium naphthalenide and samarium iodide. The authors replaced the tosyl protecting group in the uncyclized precursor **23** by a *t*-butoxycarbonyl (Boc) via formation of the disubstituted tosyl-Boc-amine and cleaving the former group with sodium anthracenilide. A number of

cyclization conditions were attempted on aldehyde **28**, but unlike the tosyl derivative, the Boc protected amine did not formed the corresponding aminal **29**.

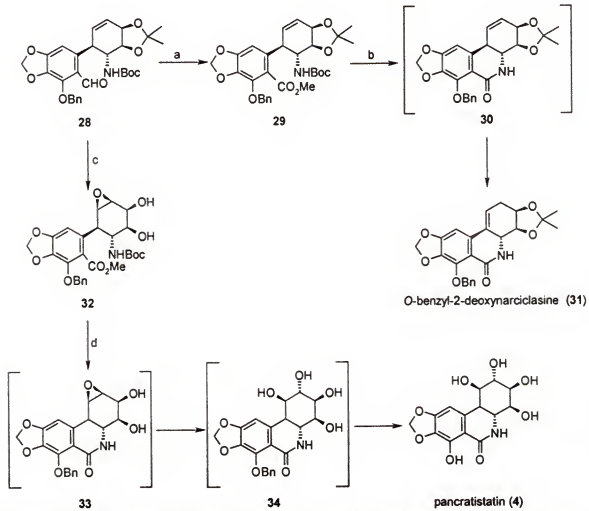


Conditions: (a) i. TBAF, THF; ii. SMEAH-morpholine, THF, -45°C. (b) i. BnBr, K_2CO_3, DMF; ii. Jones reagent. (c) i. $(Boc)_2O$; ii. Na, anthracene, DME. (d) SMEAH-morpholine, THF, -45°C.

Figure 14. Problems observed in the closure of B ring by Hudlicky *et al.*

In a new attempt, the aldehyde **28** was oxidized by means of sodium chlorite and transformed into the methyl ester **29** (Figure 15). The later was subjected mild basic conditions (K_2CO_3, MeOH) and indeed cyclized but with concomitant migration of the double bond to the C1-C10b position to form the C2-deoxynarciclasine derivative **31**, (Figure 15).

This last result made evident that the C1-C2 unsaturation must be removed before any attempts of ring B closure. Sharpless conditions were applied and the β-epoxide was



Conditions: (a) i. $NaClO_2$, K_2HPO_4, 2-methyl-2-butene, *t*-BuOH, H_2O; ii. CH_2N_2, Et_2O. (b) K_2CO_3, MeOH. (c) *t*-BuOOH, $VO(acac)_2$, benzene, 60ºC. (d) H_2O, NaBzO (cat), 100ºC

Figure 15. Pancratistatin ring B closure via amidation in aqueous media.

formed after removal of the acetonide protective group. The epoxide was then treated in the very mild aqueous conditions previously utilized by Hudlicky and Mandel to prepare D-*chiro*-inositol[41] (BzONa, H_2O, 100ºC) and a series of remarkable transformations took

place rendering pancratistatin in 50% yield. In first place the closure of the amidic B ring happened with concomitant removal of the Boc group. The epoxide was first opened with complete regioselectivity and finally the benzyl ether was cleaved liberating the alkaloid.

2.1.4.2. Hudlicky's synthesis of lycoricidine.

A disconnection of type I (Figure 10) is a powerful tool for the construction of phenanthridone architectures bearing a double bond on ring C. This was the approach utilized by Rigby in his preparation of narciclasine[21] (vide infra) and by Hudlicky and Olivo in an efficient synthesis of lycoricidine.[35]

In their preparation, Hudlicky and Olivo made use of the experience gained in the syntheses of conduramines via acylnitroso Diels-Alder cycloaddition with protected *cis*-cyclohexadiene diols.[42] They recognized the amidic precursor **35** as a conduramine derivative that could be transformed into lycoricidine by a Heck cyclization (Figure 16)

The aromatic precursor for ring A of lycoricidine was prepared from readily available piperonal (**16**). This material was brominated with bromine in carbon tetrachloride to render exclusively bromopiperonal **37** that was converted into the corresponding acid chloride **38**. Finally, the latter derivative was transformed into hydroxamic acid **39** by reaction with hydroxylamine hydrochloride and sodium hydroxide (Figure 17).

The hydroxamic acid **39** was added to a mixture of bromobenzene-*cis*-diol acetonide (**41**), obtained in two steps from bromobenzene (**40**), and tetrabutylammonium periodate. Previous studies on Diels-Alder cycloaddition to halobenzene-*cis*-diol derivatives had demonstrated that the stereochemistry is completely controlled by the

effective blocking of the α face by the acetonide group and the regioselectivity through a directing effect of the halogen atom.[42-44] Indeed, reaction of **39** with periodate generated a nitroso dienophile that was trapped by the acetonide **41** to furnish oxazine **42** as a single isomer (Figure 18).

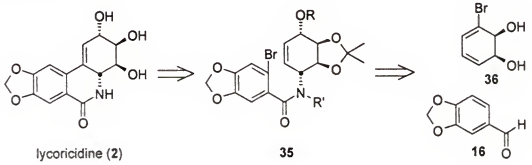
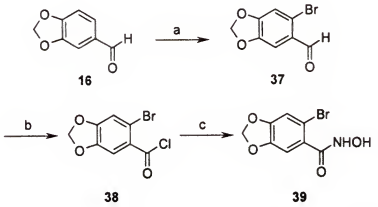


Figure 16. Hudlicky's disconnection of lycoricidine.

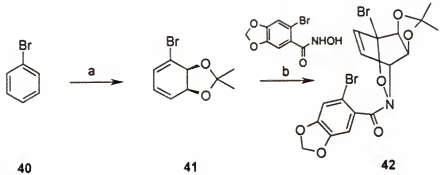


Conditions: (a) Br, CCl_4. (b) i. $KMnO_4$ or Ag_2O; ii. $SOCl_2$ or $(COCl)_2$. (c) NH_2OH, NaOH.

Figure 17. Preparation of the C Ring and Diels-Alder partner for the synthesis of lycoricidine.

Oxazines such as **42** have been cleaved smoothly to render amino alcohols by action of aluminum amalgam in tetrahydrofuran at 0°C.[45] Unfortunately, an excess of aluminum amalgam is required and this often interacts with other functionality present in

the molecule. In this case, although oxazine **42** was cleaved completely, partial debromination of the aromatic ring was unavoidable and a mixture of alcohols **43** and **44** was obtained.[46,47] To overcome this difficulty the authors prepared alcohol **45** by known methods and reduced it cleanly to hydroxycarbamate **46** before attaching the aromatic portion of the target. Latter, compound **46** was protected with isopropyldimethyldichlorosilane and the resulting carbamate acylated with bromopiperonoylchloride (**38**) to render **47**. This second route provided amide **47** (a precursor related to **35**) in sufficient amounts to attempt the final closure (Figure 19).

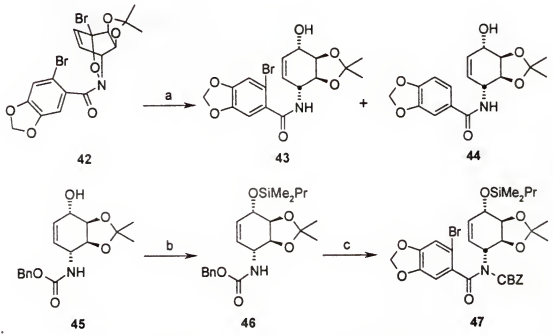


Conditions: (a) i. *Pseudomonas putida* F39/D; ii. dimethoxypropane, acetone, TsOH. (b) Bu_4NIO_4, CH_2Cl_2, r.t.

Figure 18. Coupling of the A and C rings of lycoricidine via nitroso Diels-Alder

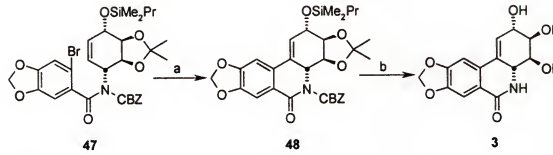
The closure was first attempted via radical cyclization (Bu_3SnH, AIBN) under different conditions but no closed products were observed. Better results were obtained with a modified Heck reaction protocol first reported by Chida *et al.* ($Pd(OAc)_2$, Tl(OAc), DIPHOS, THF).[36] This procedure produced trace amounts of the desired product but after substituting the solvent for a higher boiling ether (anisole) the desired phenanthridone **48** was obtained in 30% yield. Removal of the protecting groups was achieved by means of transfer hydrogenation followed by acid hydrolysis to afford

lycoricidine (**3**) in nine steps (Figure 20). This preparation compared very favorably with any of the previous synthesis of lycoricidine in length, simplicity, and overall yield.



Conditions: (a) Al(Hg), THF, 0°C. (b) $ClSiMe_2i$-Pr, imidazole. (c) BuLi, THF, -78°C; then **38**.

Figure 19. Preparation of the aminoalcohol precursor of lycoricidine.

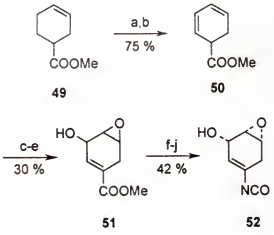


Conditions: (a) Pd(OAc)2, Tl(OAc), DIPHOS, THF. (b) i. Pd(C), cyclohexene, EtOH; ii. CF_3CO_2H, 0°C.

Figure 20. Final steps in Hudlicky's synthesis of lycoricidine.

2.1.4.3. Rigby's synthesis of narciclasine.

The first total synthesis of narciclasine was published in December 1997 by Rigby *et al.*[21] The procedure started from 3-cyclohexene-1-carboxylic acid (**49**) and produced the alkaloid in 23 steps and overall yield. The asymmetric precursor (epoxide **52**) was constructed in nine steps via resolution using a cholesterol esterase (Figure 21).



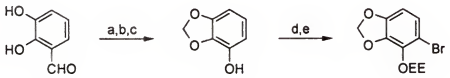
Conditions: (a) NBS, AIBN, PhH, reflux. (b) AIBN, Bu_3SnH, PhH. (c) $^1O_2$, hν, rose Bengal. (d) $(Ph_3P)_2RuCl_2$, CH_2Cl_2. (e) NaOMe, MeOH. (f) butyryl chloride, TEA. (f) cholesterol esterase. (h) TBSCl, imidazole. (i) LiOH, MeOH, H_2O. (j) DPPA, TEA, PhH.

Figure 21. Asymmetric branch of Rigby's synthesis of narciclasine.

In order to construct the aromatic portion of narciclasine (Figure 22), Rigby utilized a methodology similar to the one used in Trost's synthesis of pancratistatin.[48]

The preparation of polysubstituted phenols is not trivial because of the inherent instability of this type of molecules. Particularly, the 1,2,3-trioxygenated pattern present in many Amaryllidaceae alkaloids enhances the acidity of the molecule to a point that the methylenedioxy protection of a simple precursor such as pyrogallol becomes unfeasible.

A common alternative to get around this problem is to mask one of the hydroxy groups as a carbaldehyde that is revealed later via the Baeyer-Villiger oxidation followed by hydrolysis. In Rigby's synthesis, the *ortho* bromination of phenol **54** was attained with the aid of silver trifluoroacetate. Ishizaki described an alternative to this technique in his synthesis of (+/-)-lycoramine.[49] The bromination is performed at low temperature in toluene in the presence of *tert*-butylamine. It is believed that the actual brominating species is not bromine but *N*-bromo-*tert*-butylamine formed *in situ* before the addition of the phenol to the reaction mixture. This sequence was also useful for the preparation of other polyoxygenated alkaloids. For example, Hudlicky has utilized this procedure to prepare the A ring of morphine in a recent approach.[50,51]



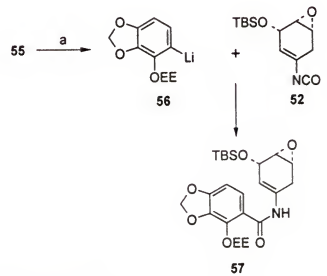
53 **54** **55**

Conditions: (a) CH_2Br_2, K_2CO_3. (b) *m*-CPBA. (c) KOH/EtOH. (d) CF_3CO_2Ag, Br_2. (e) ethyl vinyl ether, PPTS.

Figure 22. Preparation of the aromatic fragment of narciclasine.

In the coupling step of Rigby's synthesis, the asymmetric intermediate **52** was reacted with the lithiated derivative **56** to provide enamide **57** in 52% yield (Figure 23). In this way, Rigby makes the amide bond before establishing the crucial connection between C10a and C10b (disconnection I, Figure 10), as in Hudlicky's preparation of lycoricidine. The final closure was performed by an electrocyclic reaction through the imidate affording phenanthridone **59** in 46% yield based on recovered starting material

(Figure 24). The authors reported that several unsuccessful attempts to improve the yield of this reaction were made.



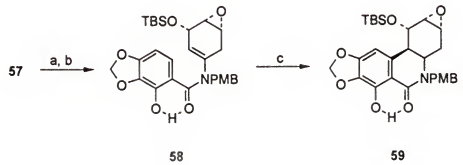
Conditions: (a) *n*-BuLi, THF, -78 °C.

Figure 23. Coupling of the aromatic and asymmetric fragments.

In order to control the stereochemistry of the closure, the ethoxyethyl group was removed and the amidic proton protected. The authors claimed that intermediate **58** is locked in position by intramolecular H-bonding. They supported the intramolecular H-bonding postulate by the observation that the best yield of phenanthridone **59** was obtained when methanol was used in the reaction. The importance of intermolecular H-bonding in such solvent was disregarded (infrared data on compound **58** in solution should provide some insight in this issue).

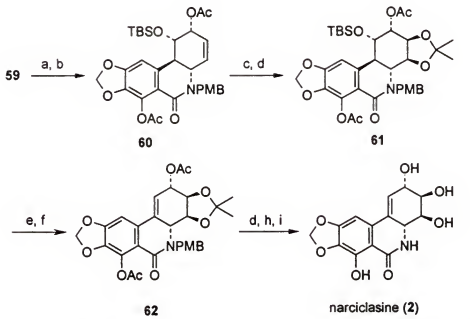
After the carbon skeleton was completed, it was necessary to adjust the asymmetric ring C in order to obtain the correct narciclasine functionalization. This

process required nine extra steps, which involve epoxide opening, elimination and dihydroxylation reactions as well as a protection deprotection sequence (Figure 25).



Conditions: (a) PMBBr, NaH. (b) PPTS, MeOH. (c) hν, PhH.

Figure 24. Closing of ring C ring via eletrocyclic reaction.



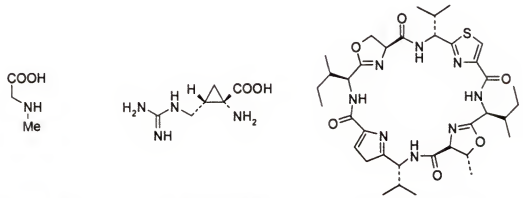
Conditions: (a) $(PhSe)_2$, $NaBH_4$, Ox. (b) NaH, AcCl. (c) OsO_4, TMNO, *t*-BuOH. (d) TsOH, $(CH_3)_2C(OMe)_2$. (e) F^-, THF. (f) Burgess Reagent. (g) K_2CO_3, MeOH. (h) *n*-BuLi, THF, O_2. (i) TsOH.

Figure 25. Elaboration of the asymmetric portion of narciclasine and final deprotection.

2.2. Morphine

2.2.1. Synthesis of Amino Acids via Chelate-Enolate Claisen Rearrangement

The study of unnatural and non-proteinogenic amino acids has been recognized as an important subarea in organic chemistry. Non-proteinogenic amino acids are substances that although being naturally occurring compounds, they are not found as constituents of proteins. Some of them, such as sarcosine (**63**), closely resemble regular proteinogenic aminoacids, while others are structurally very different from the standard set. Sometimes they happen as single monomeric structures such as the antibiotic (-)-carnosadine (**64**)[52] or as a part of oligomeric peptides as in patellamides (**65**),[53,54] a fascinating class of marine natural products (Figure 26).



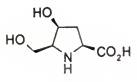
sarcosine (**63**) (-)-carnosadine (**64**) patellamide A (**65**)

Figure 26. Examples of naturally occurring non-proteinogenic amino acids.

The term "unnatural" has been applied to almost any amino acid that has a α-amino function to the carboxylate and bears a side chain group that is different from the ones present in the natural amino acids. This class includes sophisticated structures

containing unsaturations or additional chiral centers (Figure 27). The term is also often used to refer to the enantiomers (*d* series) of the naturally occurring compounds.

Both non-proteinogenic and unnatural amino acids have received attention as synthetic targets for several reasons. In the first place, they are relevant drug candidates for the pharmaceutical industry. They may not only be often bioactive by themselves, but



66 67

Figure 27. Examples of unnatural amino acids.

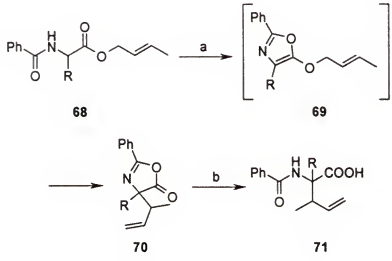
they can also substitute a natural (or proteinogenic) analog inside a bioactive peptide in order to change its properties or make it more resistant to enzymatic hydrolysis. This is a relevant fact when considering combinatorial methods of synthesis. Despite the fact that the generation of huge libraries of small peptides produced many bioactive substances; most cannot be used as drugs because of their short-half life under *in vivo* conditions.

In addition to their pharmacological potential, amino acids are also useful synthons. Many secondary metabolites, and particularly alkaloids, are biosynthetically derived from amino acids. Even without an involvement of a biomimetic route, amino acids are interesting starting materials for alkaloid synthesis. They, by definition, contain nitrogen that is also an essential constituent of alkaloids. They are easy to functionalize and, if optically pure, they can translate or incorporate that asymmetry into the target.

The preparation of new (unnatural) amino acids in an optically pure fashion therefore provides a wide array of new synthons for alkaloid synthesis.

2.2.1.1. Previous work.

The first report on preparation of amino acids by a Claisen rearrangement was the work of Steglich.[55,56] He discovered that *N*-benzoyl α-amino acid esters of type **68** will react under dehydrating conditions to form the corresponding oxazoles **69**. This molecule is not isolated and it rearranges to the more stable oxazolones **70**. Hydrolysis of the heterocycle renders a β-substituted amino acid of type **71** in excellent yield (Figure 28).



Conditions: (a) PPh_3, CCl_4. (b) H_3O^+, Δ.

Figure 28. Synthesis of amino acids via oxazole rearrangement

The oxazole intermediate **69** can be viewed as a trapped enolate of fixed geometry. This is a crucial factor in order to achieve predictable stereoselectivity in the Claisen rearrangement. Indeed, the final stereochemical outcome of the rearrangement will depend on both the geometry of the enolate and the transition state (chair or boat). In

a non-trapped enolate, these two variables will result in four possible pathways of reactions towards the two expected products (**79** and **80**) (Figure 29). By "locking" the enolate into the oxazole structure, the Steglich method eliminates one variable of the system leaving only two feasible pathways, each dictating a different product. Unfortunately, this elegant strategy suffers an important limitation. Whenever the R group is a hydrogen oxazolones **70** rapidly epimerize in the reaction conditions. In fact, for this type of substrates, the rearrangement takes place with little or no final stereoselectivity.

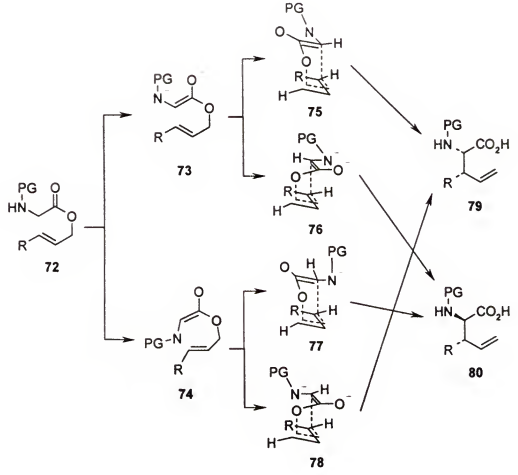
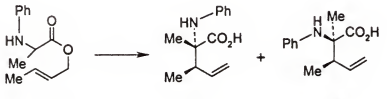


Figure 29. Four possible pathways of reaction in a enolate Claisen rearrangement.

In 1982, the work of Steglich was reviewed by Bartlett[57] and compared to the standard Ireland conditions.[58] According to Bartlett the oxazole method can compare favorably with the classical silylketene acetal approach in terms of yield. The stereoselectivity observed was good for β-disubstituted amino acids that cannot undergo racemization but poor or no stereoselectivity was observed in the monosubstituted rearranged products. In some cases, the oxazole rearrangement showed poor selectivity even for disubstituted amino acids. This indicated that a mixture of boat- and chair-like transition states coexisted in the reaction coordinate. Bartlett found this surprising since the chair-like transition state is generally preferred for acyclic substrates.[59] Interestingly, the silyl enolate method showed a better selectivity in the same system (Figure 30).



81 **82** **83**

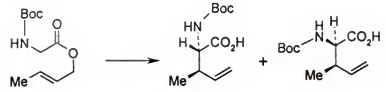
enolate:	4	1
oxazole:	1.5	1

Figure 30. Bartlett comparison between the oxazole and enolate methods for identical substrates.

Bartlett also studied the effect of different solvents and bases in the rearrangement.[57] The results are summarized in Table 4. The use of a less polar solvent like ether showed a slight increase in selectivity but accompanied a decrease in overall yield. The addition of a stronger coordinating cosolvent such as HMPT decreased both

yield and selectivity (Table 4). The presence in the reaction mixture of a strongly chelating cation (Mg^{2+}) increased the selectivity, but again decreased the yield. In principle, the results obtained can be explained by postulating the combination of a *cis* enolate and a chair transition state or a *trans* enolate and a boat transition state (Figure 29). Because of the well-established preference for the chair,[59] Bartlett proposed that the formation of a bident chelate between the enolate and the lithium ion was the factor controlling the stereochemistry of the reaction. The addition of HMPT might have disturbed this chelation, thus decreasing the stereoselectivity.

Table 4. Influence of the addition of cosolvents and Lewis acids in the Ireland-Claisen rearrangement of amino esters.



84 **85** **86**

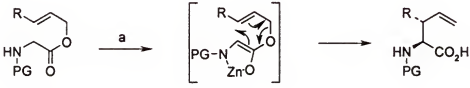
Conditions	Yield	Ratio of **85/86**
Standard	60-65	9
Diethyl ether	45	10
20 % HMPT/THF	51	4
1.1 eq. of $MgCl_2$	42	10

In summary the work of Steglich and Bartlett proved the feasibility of the rearrangement and showed the advantages and limitations of fixing the enolate geometry via formation of a heterocyclic ring. Some of the experiments by Bartlett suggested that

metal chelation might improve the selectivity of the reaction and become an adequate substitute for the oxazole method.

2.2.1.2. Kazmaier chelated-enolate Claisen rearrangement.

In 1994, Kazmaier reported a variation of the classical enolate Claisen rearrangement that proved to be useful in the synthesis of unnatural amino acids.[60,61] The initial addition by Kazmaier to the previous technology was the confirmation that enolized amino esters can form a bidental chelate with a Lewis acid (Figure 31). Instead of making the silylketene acetal, Kazmaier allowed the chelated enolates to warm up and observed that they cleanly rearranged at -15 ºC with excellent yield and diasteroselectivity. A number of metal counterions and Lewis acids were evaluated and the results in both diastereoselection and yield were excellent (Table 5). The enolates formed were less reactive (hence more stable) than the lithium enolates but more reactive than the silylenolethers allowing the reaction to occur below room temperature.



72 87 80

Conditions: (a) LDA (2.2 eq.), $ZnCl_2$ (1.1 eq.), THF, -78 to -15ºC, then acidic workup
Figure 31. Kazmaier chelated-enolate Claisen rearrangement.

The stability of the chelate assures that only the *cis* enolate will react in the rearrangement, thus reducing the number of variables in the system from two to one in the

same way as the Steglich oxazole rearrangement mentioned above. Indeed, the stereochemistry of the products (in the context of diasteroselectivity in the rearrangement) will depend only on the conformation of the transition state. The product alternatives arising from the *trans* enolate can be discarded making the analysis of the products and reaction stereocontrol simpler.

Table 5. Kazmaier results in the chelated-enolate Claisen rearrangement using various salts as chelating agents.

Lewis acid	% yield	% de
$CaCl_2$	73	96
$ZnCl_2$	90	90
BCl_3	93	86
$MgCl_2$	85	82
$Al(OiPr)_3$	75	80
$Ti(OiPr)_4$	50	80
$SnCl_2$	88	50

The methodology has been expanded from simple acyclic amino ester to cyclic compounds. The results of the rearrangement in cyclic and especially six-membered ring substrates were of special interest and are summarized in Table 6.

Since the geometry of the transition state determines the disteroselectivity of the rearrangement it is of distinct importance to study the particularities of the cyclic case. In

general, it is accepted that a chair-like transition state is predominant for acyclic substrates (*vide supra*), but a boat-like transition state has been often discussed to explain the results obtained with cyclic substrates.[62] The results of Kazmaier for five, six, seven and eight membered rings indicated a predominance of the boat-like transition state. The alternative route to the observed diasteroselectivity would require the formation of an unchelated (*trans*) enolate and a chair transition state. Such combination was unlikely in the reaction conditions.

Table 6. Summarized results for cyclic substrates in the helated-enolate Claisen rearrangement.

88 **89**

n	% Yield	% de
1	79	60
2	83	80
3	73	84
4	57	72

Kazmaier has thoroughly studied this reaction and expanded the methodology to more interesting compounds such as α-alkylated amino acids[63] and several other sterically demanding targets.[64,65] Following a previous report by Corey[66] on an asymmetric version of the regular Claisen rearrangement, Kazmaier has studied the

asymmetric catalysis of his reaction by chiral amines.[65,67-71] The conditions for the rearrangement are mild and both enantiomeric amino acids are accessible (Figure 32). Finally, the same author has expanded the methodology to the modification of small peptides (2 or 3 units) hoping to develop a procedure to incorporate allylic chains on larger peptides in a diasteroselective fashion.[72,73] The procedure worked well in terms of yield, but the stereoselectivity was far from optimal. A ratio of 2.2/1 was obtained in the best cases and the outcome was in general difficult to predict. Kazmaier has explained this result in terms of the diversity of chelation sites present in the substrates. He proposed that the chelation between the metal ion and other Lewis bases present in the molecule (aside from the reaction site) degraded the transfer of stereochemical information from the peptide molecule to the products.

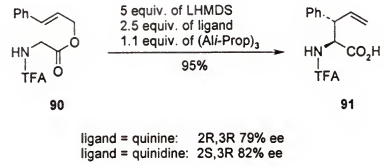


Figure 32. Asymmetric version of the chelated-enolate Claisen rearrangement.

In summary, a new variation of the classical enolate Claisen rearrangement has been developed by Kazmaier. The methodology proved to be useful for the synthesis of simple amino acids from the corresponding unrearranged esters in good yields and in a diasteromeric excess above 80% in all cases. The author has developed an asymmetric

version of the reaction and has attempted to expand the methodology to more complicates substrates. The stereochemical outcome of the reaction was predictable (at least for the simpler substrates) and the reaction seemed to follow well-established pathways. The chair-like transition state predominated for acyclic substrates while the boat-like transition state dictated the stereochemistry in the cyclic cases.

2.2.2. Morphine Syntheses via Sigmatropic Rearrangements

According to the available records, the relationship between opium and the human beings started in ancient Middle Eastern civilizations about 3500 years ago.[74] Since then, the potent bioactivity of morphine and its congeners has been an important issue that has transgressed the frontiers of medicine and has become a social and political factor as well. The tremendous analgesic power of morphine, that is still unrivaled today, has allowed the drug to survive as an essential member in every pharmacopoeia of the world despite the undesired euphoric and addictive side effects. In this perspective, it is not surprising that the chemical community had been attacking the difficult problem of morphine synthesis since the early days of modern organic chemistry. In fact, morphine had been a synthetic target even before the structure of the molecule was elucidated. These efforts have resulted in more than twenty total synthesis of morphine and a number of additional synthetic approaches. Although such work has greatly contributed to the development of alkaloid synthesis, to date no synthesis has been able to compete economically with the simple procedure of extraction and purification of the alkaloid from *Papaver somniferum*. Motivated by the desire to emulate or even surpass Mother Nature's preparation, several groups worldwide continue to work on practical synthesis of morphine as is revealed by the routine occurrence of reports in the field. A comprehensive review of seventeen

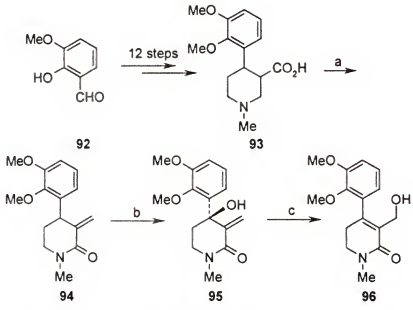
syntheses of morphine published before 1995 has been provided by Hudlicky *et al.*[74] Since then several other syntheses and approaches have been published. Despite the massive amount of work displayed in the area, many preparations follow a few common motifs. Ultimately, many relate to the pioneering work of Gates who published the first total synthesis of morphine in 1952.[75] In fact, a known issue in the morphine field is the frequent occurrence of formal syntheses that intercept advanced intermediates from Gates' or Ginsburg's[76] original preparations.

Although a wide set of synthetic tools has been applied to the morphine problem, the use of sigmatropic rearrangements has not been thoroughly studied until relatively recently. Only the preparations of Rapoport,[77] Parsons,[78,79] and more recently Mulzer[80] have relied on this type of reactions. Interestingly, the three syntheses made use of the rearrangement for the same purpose, installing the quaternary center at C13 (morphine numbering), while transferring the stereochemistry already present in the starting material to that position. This differs from our approach where the goal was to transfer the stereochemistry from C5 to the consecutive centers C9 and C14.

2.2.2.1. Rapoport synthesis.

Although Rapoport had developed wide experience in the morphine field before targeting the total synthesis, his preparation suffered from a number of stereochemical problems and resulted in a lengthy preparation. The author finally chose to intercept Evans route[81] and opted for a formal synthesis of morphine. As mentioned above, a sigmatropic reaction (ortho-ester Claisen rearrangement) was utilized to introduce the quaternary center at C13. In order to arrive at the precursor for the rearrangement, Rapoport constructed amino acid **93** that rearranged in the presence of acetic anhydride to

lactam **94** (Figure 33). The authors have extensively studied this rearrangement and applied it to a diverse group of β-amino acids.[82] Benzylic oxidation of **94** followed by reaction with formic acid rendered, after allylic migration and hydrolysis, precursor **96**. Overall, the required alcohol was prepared in seventeen steps from *ortho*-vanillin (**92**).



Conditions: (a) Ac_2O. (b) SeO_2, PhCl, 100 °C. (c) i. HCO_2H; ii. K_2CO_3, MeOH.

Figure 33. First steps in the Rapoport synthesis. Stereospecific preparation of a rearrangement precursor.

Upon reaction with an orthoester, alcohol **96** formed acetal **97** which cleanly rearranged to ethyl ester **98**. This compound contained the required quaternary center at C13 as well as the complete C ring with an adequate pattern of substitution. Ring B was also present and so was the required *N*-methyl group found in morphine. Ring A was incipient in this structure and still necessitated further elaboration. After several steps and many disappointing results, Rapoport decided to intercepted the advanced Evans

intermediate **99**.[81] Evans synthesis was also formal since it terminated at the classic Gates' ketone **100** (Figure 34).[75] In that sense, it can be said that Rapoport's preparation was doubly formal.

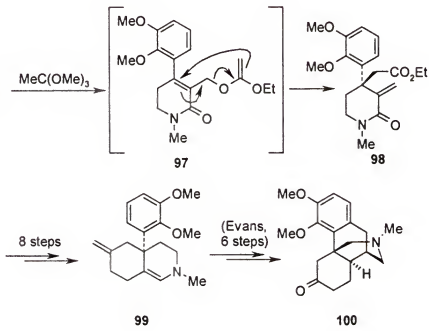
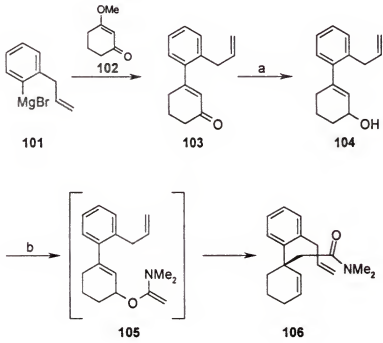


Figure 34. Orthoester Claisen rearrangement and elaboration to an Evans intermediate.

2.2.2.2. Parsons' synthesis.

In 1984, Parsons and Chandler[78] reported an elegant synthesis of morphinan **107** (Figure 35). In their approach, the authors quickly assembled rings A and D by 1,2 addition of Grignard compound **101** to six-membered ketone **102**, followed by hydrolysis. The resulting ketone was reduced under Luche conditions to render allylic alcohol **104**. The latter was reacted with dimethylacetamide dimethyl acetal to form allylic acetamido acetal **105** that was not isolated since it rearranged *in situ* to render the Eschenmoser-

Claisen amide **106** (Figure 35). In this way, the Claisen rearrangement was used to introduce the quaternary center at C13. The stereochemistry was not crucial at this point because this was the first asymmetric center set, and this study was being executed in the racemic series.

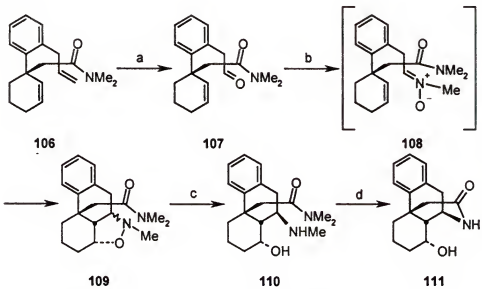


Conditions: (a) i. $NaBH_4$-$CeCl_3$. (b) dimethylacetamide dimethyl acetal, PhMe, reflux.
Figure 35. Parsons' original approach to the morphine skeleton.

Ring B was closed by a very interesting sequence. Amide **106** was ozonized and the resulting aldehyde **107** treated with *N*-methylhydroxylamine. The dipolar intermediate **108** was not isolated. Under the reaction conditions (refluxing benzene) **108** generated isoxazolidine **109** in 72 % yield by intramolecular cycloaddition. This material showed correct relative stereochemistry at C14 but a complete lack of stereoselectivity at C9, therefore the epimers were separated chromatographically. The N-O bond of the β

isomer was cleaved by catalytic hydrogenolysis to produce amino alcohol **110** which was cyclized simply by heating the corresponding hydrochloride salt under vacuum. Lithium aluminum hydride reduction of the resulting hydroxyamide rendered morphinan **111** in 2.1 % overall yield (Figure 36).

The merits of this procedure are the elegance of the sigmatropic rearrangement and the efficient closure of ring B by [3+2] dipolar cycloaddition. Although the latter did not achieved double selectivity (and therefore did not solve the difficult problem of the relative configuration of C9 and C14) it did set one center correctly (relative to C13) and interestingly introduced an alcohol group at C8. This group could later eliminate to establish the olefin at C7-C8 in a complete synthesis of morphine, or can be of use in the generation of synthetic analogs.



Conditions: (a) O_3. (b) MeNHOH, PhH, reflux. (c) W2 Raney Ni, H_2. (d) i. $HCl_{(g)}$, Δ; ii. $LiAlH_4$

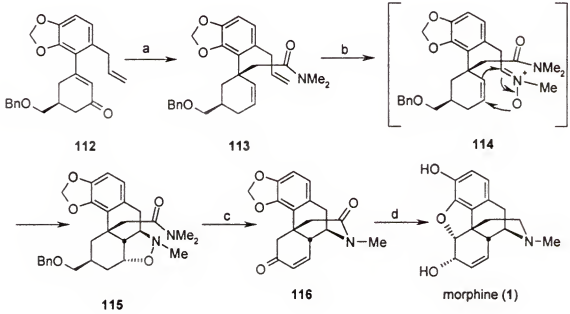
Figure 36. Parsons' approach to morphinan **111** via 2,3-dipolar cycloaddition.

According to a review by Parsons[79] the 1984 approach has crystallized in a previously unpublished total synthesis of morphine. In this second-generation synthesis, Parsons started from the chiral ketone **112**, prepared analogously to the model compound **104**. Reduction of the ketone from the less hindered side of the molecule followed by Eschenmoser-Claisen rearrangement generated amide **113** as a single isomer (Figure 37). Dipolar cycloaddition furnished isoxazolidine **115** (the stereoselectivity of this reaction is depicted as reported in Parsons' paper). Hydrogenolysis cleaved the isoxazolidine and debenzylated the hydroxyl group and the nitrogen ring (D ring) was closed according to the previously established conditions. The resulting morphinan was treated with nitrophenylselenyl cyanide to give a selenide that was oxidized *in situ* to generate enone **116**. Removal of the methylenedioxy group and closure of the E ring was achieved synchronically in a carbene reaction. Reduction of the resulting amide with lithium aluminum hydride rendered free morphine in 6% yield from the isoxazolidine **115**. This interesting and potentially powerful sequence is only briefly described in the review and curiously has never been published in detail.

2.2.2.3. Mulzer synthesis.

In a recent series of papers,[80,83,84] Mulzer has studied the hydrophenanthrene route to morphine synthesis. This strategy consists in the preparation of a substituted hydrophenanthrene core where the ethylamino bridge could be mounted subsequently. A fundamental issue to solve in any approach of this type is the introduction of the quaternary center at C13 onto the complete phenanthrene subunit. According to Mulzer two basic strategies can overcome this botleneck; conjugate addition of a cuprate to an

unsaturated ketone[80,83] or a Claisen rearrangement of the corresponding ether derived from allylic alcohol **119**.[80,84]



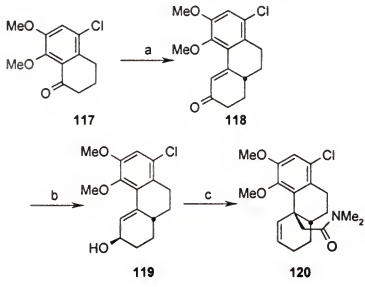
Conditions: (a) i. $NaBH_4/CeCl_3$; ii. $MeC(OMe)_2NMe_2$, 49%. (b) i. OsO_4; ii. $NaIO_4$; iii. MeNHOH, 30%. (c) i. H_2, $PdCl_2$; ii. $HCl_{(g)}$, Δ; iii. *p*-$NO_2C_6H_4SeCN$, Bu_3P, then H_2O_2; iv. O_3, Ph_3P. (d) i. $CuBr_2$, MeCN, then K^+t-BuO^-; (ii) $LiAlH_4$

Figure 37. Parsons' final steps in the synthesis of morphine.

The preparations started from the known tetralone **117**[85] that was converted into unsaturated ketone **118** via Robinson annulation this compound was also used for the conjugate addition studies). The racemic ketone **118** was resolved by means of chromatography on a chiral support and the desired enantiomer reduced with DIBAL to afford alcohol **119** as a β/α : 82/18 mixture. Probably inspired by the work of Parsons (vide supra), Mulzer reacted **119** with *N,N*-dimethylacetamide dimethyl acetal that

cleanly rearranged in an Eschenmoser-Claisen reaction to render the advanced amide **120** (Figure 38). The use of the Eschenmoser variation seems better justified here than in Parsons approach since the incorporated nitrogen atom was the one that would eventually become part of the D ring. Actually, amide **120** had all the atoms that form the morphine skeleton except the C6-hydroxyl, which could be easily restored.

Mulzer chose to close the oxygen ring (E-ring) before completing ring D. The amide was fully reduced and the resulting alcohol was transformed into a *N*-sulphonamide by a modified Mitsunobu protocol. Epoxidation with dimethyldioxirane rendered **122** in a β/α : 8.5/1 ratio. In a remarkable transformation, concerted ring

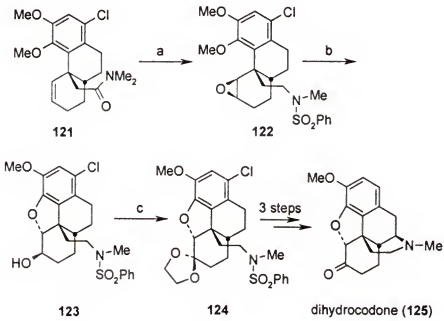


Conditions: (a) i. HCOOMe, NaOMe, PhH; ii. MVK, Et_3N, MeOH, then KOH, dioxane. (b) i. chromatographic resolution , ii. DIBAL, THF. (c) *N*,*N*-dimethylacetamide dimethyl acetal, PhMe, reflux.

Figure 38. Mulzer's preparation of an advanced intermediate via Eschenmoser-Claisen rearrangement.

closure, restoration of the C6-hydroxyl group and demethylation was achieved by treating β-**122** with trifluoroacetic acid at room temperature. The resulting alcohol **123** was

dechlorinated, oxidized and protected as the ethylene ketal **124**. Finally, this compound was further elaborated to dihydrocodone (**125**) by previously reported procedures (Figure 39).[83]



Conditions: (a) i. $LiBHEt_3$, THF, r.t.; ii. $PhSO_2NHMe$, ADDP, Bu_3P, r.t.; dimethyldioxirane, CH_2Cl_2, 0 °C. (b) TFA, THF, r.t. (c) i. H_2, Pd/C, Et_3N, MeOH, r.t.; ii. Swern oxidation; iii. TMSCl, $(CH_2OH)_2$, CH_2Cl_2, r.t.

Figure 39. Formation of the E ring and final elaboration to dihydrocodone (**125**).

In summary, the Claisen rearrangement has been a useful tool for the introduction of the crucial quaternary center in three different morphine formal synthesis, but comparable results have been obtained with alternative methodologies. In particular Mulzer has compared the Claisen rearrangement vs. the cuprate addition to conjugated ketones and found the latter superior at least for his type of approach. To the best of my knowledge, the Claisen rearrangement has not been used to generate or transfer

stereochemistry to any other portion of the morphinan skeleton besides the C13 center. Nevertheless, these examples prove that [3,3]-sigmatropic rearrangements and specifically the Claisen rearrangement are valuable tools in the morphine field. This precedent added to the recent advances in amino acid synthesis via Claisen rearrangement support a strategy that utilizes a chiral unnatural amino acid as a key intermediate in an approach to the morphine skeleton.

CHAPTER 3
RESULTS AND DISCUSSION

3.1. Isolation and Identification of New Metabolites

In the general introduction chapter, we outlined a synthetic strategy towards two classes of alkaloids (Figure 2). In this dissertation we intended to prove that it is possible to design a strategy, disconnecting the target to the ideal *cis*-cyclohexadienediol, even if the metabolite is not known at the time of the plan. The biotransformation techniques have reached a degree of maturity in which almost any required synthon can be custom prepared to be exploited by the synthetic chemist.

To support this statement we attempted the preparation of a series of previously unknown *cis*-cyclohexadienediols that will later be used in the synthetic projects. In this way, we evaluated the ability of toluene dioxygenase to dihydroxylate several aromatic compounds. The testing of a new substrate and subsequent scale up of the reaction followed a detailed protocol given in the Experimental Part of this dissertation. An aspect that merits mention in this section is that the reactions were performed exclusively as "whole cell" biotransformations. This means that actual living organisms (bacteria) were employed. This contrast with the "isolated enzyme" biotransformations where no real living entity is involved. Although both procedures have their own advantages and disadvantages, the "isolated enzyme" method is not economically adequate whenever expensive cofactors are required in the enzymatic reactions involving oxido-reductases.

Since toluene dioxygenase is a NAD^+ dependent enzyme, carrying out isolated enzyme experiment is only justified in particular cases, mainly metabolic studies. Nevertheless, in order to obtain multigram amounts of a homochiral starting material "whole cell" oxidations are the best choice.

There are generally two types of bacteria that are used to oxidize aromatic compounds to cyclohexadiene-*cis*-diol. The organisms most commonly used are mutants of the wild-type strain that have lost the ability to dehydrogenate *cis*-diols, and recombinant strains of *Escherichia coli* that contain the cloned dioxygenase genes. Consequently, there are two procedures to be followed in terms of utilizing these organisms to produce cyclohexadiene-*cis*-diols. Both procedures can be easily performed with minimum skills in microbiology.

The first procedure involves the use of blocked mutants in which the enzyme synthesis must be induced by a known aromatic inducer (for *P. putida* F39/D this might be toluene, chlorobenzene, bromobenzene or other simple monocyclic aromatic compound). If the inducer is also the substrate to be converted to a cyclohexadiene-*cis*-diol the procedure is simple. The mutant is grown in a mineral salts medium which provides the required amount of inorganic elements (N, P, Mg, Fe, etc.) and an organic substrate that does not repress the synthesis of the dioxygenase (usually pyruvate or glucose). The aromatic compound can usually be added at the start of the growth. The accumulation of cyclohexadiene-*cis*-diol is monitored spectrophotometrically until the biotransformation ceases. The bacterial cells are then removed and the clear supernatant extracted with acid-free ethyl acetate.

Variations of this procedure are used when the substrate does not induce the dioxygenase synthesis. The mutant is grown in a mineral salt medium with pyruvate or glucose in the presence of an inducing substrate as described above. Following the induction period, a new substrate is added which, if recognized by the enzyme, is oxidized to the corresponding diol. The final fermentation broth contains the metabolites derived from the inducer and the substrate and thus such a process necessitates a separation. Alternatively, the cells might be separated from the broth after induction and resuspended in fresh medium before addition of the second substrate.

The second procedure is slightly more complex to execute but gives potential for higher cell and product yields and is ideal for testing new compounds as substrates for oxidation. It relies on the use of the recombinant organism in which transcription of the genes encoding the corresponding dioxygenase enzyme is initiated by exposure to low concentrations of non-aromatic inducer, in most cases isopropyl-β-D-thiogalactoside (IPTG). The cells are allowed to grow and synthesize the dioxygenase before the introduction of a substrate to be oxidized. Separation problems are avoided but the procedure requires the use of a fermenter with carefully regulated oxygen levels, temperature, pH, CO_2 release, and nutrient/substrate feeds.

3.1.1. Enzymatic Dihydroxylation of 1-Bromo-2,3-difluorobenzene

The biooxidation of substituted bromobenzenes, chlorobenzenes, and fluoroaromatic compounds is known to furnish 2,3-diols,[86-89] and several studies are available on the oxidation of other disubstituted arenes.[90-92] Whereas trisubstituted aromatics have not been as extensively studied, several examples of biooxidation of these compounds can be found in the literature.[89,93,94] In an effort to expand the scope of

toluene dioxygenase oxidation and to learn about the limits and specificity of the enzyme, we performed the biooxidation of a trisubstituted fluorine-containing aromatic. These study complements the one performed by Hudlicky and Stabile on fluorinated *m*-disubstituted substrates. Such fluorinated compounds can be useful for studying the complete metabolic pathway of the degradation of aromatic compounds by toluene dioxygenase and in the preparation of fluorinated carbohydrates for medical applications.[95] In a recent example,[96] the biooxidation of fluorobenzoic acids has been studied by direct analysis of the crude cell-free centrifugate by ^{19}F-NMR. Optically pure fluorine-containing diene diols may find use in the preparation of fluorodeoxysugars and inositols, compounds that are useful in evaluating metabolic pathways.[95,97,98]

126

127

128

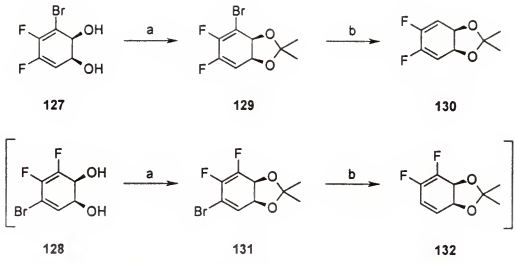
Figure 40. 1-Bromo-2,3-difluorobenzene and its two possible metabolites.

Compound **126** was oxidized by toluene dioxygenase according to both procedures described above. In the oxidation of arene **126** with *Pseudomonas putida* F39/D, only compound **127** (together with 3-methyl-1,2-cyclohexadiene diol from biooxidation of toluene) was recovered. The isolated yield was 50 mg/L, and the melting point after recrystallization (ethyl ether) was 104.5-105.5 °C. Substrate **126** was also

oxidized on large scale by means of *E. coli* JM109 (pDTG601A). In this case, metabolite 127 was obtained with a yield of 0.7 g/L as the only product.

Whenever a di- or tri-substituted aromatic is subjected to enzymatic dihydroxylation there is a question about the regiochemistry of the oxidation. It is generally accepted that the biggest substituent on the aromatic ring "directs" the reaction and the diol is formed at C2 and C3 relative to the directing group. This selectivity is not absolute and often mixtures of isomers are obtained. *Meta-* and *ortho-*substituted benzenes can produce a mixture of regioisomers while *para*-substituted analogs render mixtures of enantiomers. This fact, far from being a limitation of the method has been utilized to produce diols of unusual structure,[99] and the first synthesis that exploited this technology has been completed in 1999.[100]

In general, fluorine is a very poor "directing group" because of its small size, but the presence of two fluorine atoms in the molecule might have produced unexpected results. In principle, two possible metabolites were attainable in the biooxidation of **126**;diols **127** and **128**. We detected only one compound whose complex NMR spectra (in view of several F-H couplings) suggested structure **127** but was not enough to confirm it. A simple synthetic transformation allowed confirmation of the structure by chemical means. In a voluntary act of "chemical vandalism" (*i.e.*, the willful destruction of chirality), the derivative **129** was transformed into the *meso* compound **130** by reductive dehalogenation (Figure 41). The alternative structure **128** was then ruled out, because, if subjected to the same dehalogenation procedure, it would have yielded the optically active compound **132**, therefore, the structure of the bromodifluorodiol obtained from the biotransformation was assigned as **127**.

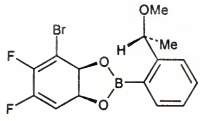


Conditions: (a) DMP, acetone, r.t., 0.5 h., 95%. (b) *t*-BuLi, THF, -78 °C, 15 min, then MeOH, 65 %.

Figure 41. Chemical proof of the regiochemistry of substituted benzene **126**.

When a new metabolite is isolated the absolute configuration of the chiral centers must be established and the optical purity determined. For simple monosubstituted diols the absolute stereochemistry has always found to be as shown in Figure 1 and the enantiomeric excess calculated as above 98% but this selectivity sometime decreases for more substituted diols. Since generally there is not access to of both enantiomers of a microbial metabolite chromatographic methods are not reliable for optical purity measurement. A reliable technique for determining the absolute stereochemistry and measuring the enantiomeric excess is to transform the newly isolated metabolite into another compound of confirmed known absolute stereochemistry and compare the corresponding optical rotation values. The sign of the recorded number indicates the absolute stereochemistry and the ratio of the literature and newly recorded values provides a number for the enantiomeric excess. Optical methods such as circular

dicroism are available for indicating the absolute stereochemistry of a compound but they cannot be considered absolute proofs. Mosher esters, which are popular derivatives used for enantiomeric excess determinations have been found difficult to apply for these metabolites but recently Resnick and Gibson[101] have reported a new NMR method that can be used for this class of compounds. The method was successfully utilized to determine the absolute stereochemistry and enantiomeric excess of diol metabolites present in small amounts (*e.g.*, 2 mg). For a series of monosubstituted arene *cis*-diols of known optical purity and absolute stereochemistry, it was observed that ^{1}H-NMR analysis of the corresponding chiral boronate esters provided well-resolved diasteromeric methyl and methoxy signals if a suitable solvent was used.



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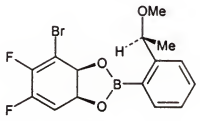

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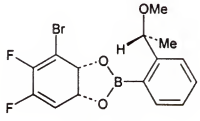

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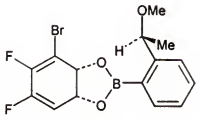


135

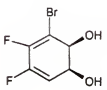
Figure 42. Structures of the four possible diasteromeric boronic esters that could have been found. Only compounds **133** and **134** were detected.

This approach has been studied in Hudlicky's laboratory by Dr. Mary Ann Endoma[102] and applied to a series of metabolites, among them compound **127**. With the *R*- and *S*- boronic acid in hand, both diasteromeric ester derivatives of **127** were prepared (Figure 42). The ^{1}H-NMR signals of either diasteromeric esters of **127** indicated the "normal" absolute stereochemistry. Indeed the chemical shifts of both examined esters followed the trend of structurally related diols of well established absolute stereochemistry. As shown in Table 7, the signals corresponding to the methoxy group in the *S*-boronic ester derivatives were found systematically downfield from the corresponding signals of the *R* derivative. A trend also was found for the methyl signals but in the latter case the *S*-ester signals were shifted upfield from the corresponding *R* isomer. Since metabolite **127** followed this trend it was assigned the "normal" 1*S*, 2*S* configuration at the chiral centers. This method does not constitute an absolute proof, especially in light of the fact that the enantiomer of **127** is not available and it cannot be checked to verify if it reverses the trend. Still these results suggested that the stereochemistry of the metabolite should be as drawn. In addition, since there were no peaks observed in the NMR corresponding to the other diasteromeric boronic ester the enantiomeric excess must be above 95%.

3.1.2. Enzymatic Dihydroxylation of Oxygenated Biphenyls

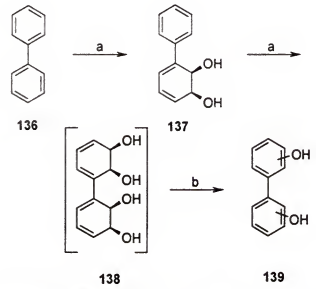
As an essential part of our synthetic strategy we necessitated to prepare a series of diols derived from highly oxygenated biphenyls (Figure 2). We intended to evaluate and compare the direct oxidation of a substituted biphenyl against the coupling of a halogenated diol and an oxygenated benzene.

Table 7. Characteristic chemical shifts of *R*- and *S*-boronic esters of microbial metabolites (the values for entries a, b are literature values).[101]

Entry	Diol	Data for the corresponding (R or S) boronate ester derivative. Methoxy signal (ppm)		Methyl signal (ppm)	
		S	*R*	*S*	*R*
a		3.175	3.159	1.551	1.580
b		3.207	3.184	1.573	1.579
c		3.144	3.121	1.519	1.544

Although strains of *Beijerinckia* and *Pseudomonas* are known to oxidize biphenyl (**136**) to 3-phenyl-(1*S*,2*R*)-3,5-cyclohexadiene-1,2-diol (**137**),[92,103,104] few substituted biphenyl diol metabolites have been isolated and fully characterized. Most of the work on this class of compounds has been done on mono- and polychlorinated diols derived from the biooxidation of PCB's.[105,106] In addition, Furukawa and collaborators have recently reported that strains of *Pseudomonas* are capable of oxidizing biphenyls bearing a wide range of substituents on one ring. Specifically, they found that dihydroxylated biphenyl (**137**) could be oxidized to the corresponding bis-diols and that the second

oxidation took place exclusively on the non-substituted aromatic ring (Figure 43).[107] Unfortunately, the authors did not attempt to isolate tretrol **138**. Instead, they chose to willfully aromatize all diene diols present in the broth by addition of acid and recover the corresponding phenols. Still, the detection of bis-phenols **139** provided indirect proof of the formation of **138**. This interesting double-oxidation can be of potential application in organic synthesis if the structure of the bis-diol **138** can be confirmed and if its recovery from the aqueous phase is feasible.



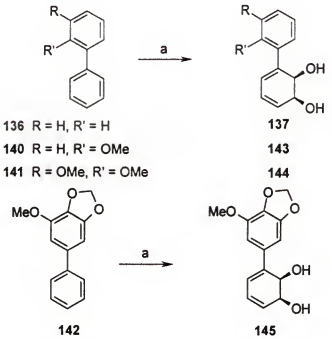
136 **137** **138** **139**

Conditions: (a) *P. pseudoalcaligenes* KF707C1. (b) aq. HCl.

Figure 43. Formation of bis-phenols by biphenyl dioxygenase dihydroxylation followed by acid-catalyzed aromatization.

As a part of the exploration of a practical approach to the morphinan and phenanthridone alkaloids skeleton from biphenyl diols, it became necessary to investigate the best method for preparation of biphenyl diols **137**, **143**, **144**, and **145** (Figure 44).[13] The hypothesis to test at this point was if the direct oxidation of biphenyls was a suitable

strategy or if it was obligatory, or are least convenient, to rely on a different approach. These types of compounds can also be prepared by palladium catalyzed cross-coupling reactions of an iodo- or bromo-diol derivative and a properly substituted boronic acid or trialkylstannane compound.[108] Moreover, even if the direct oxidation of biphenyls resulted in a better method of synthesis, the palladium mediated coupling provided an optimum route for confirming the structure and absolute stereochemistry of the new metabolites.

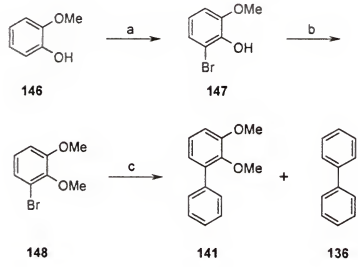


Conditions: (a) *E. coli* JM109 (pDTG601A).

Figure 44. Oxidation of synthetically relevant biphenyls with toluene dioxygenase.

The first step in the project was the preparation of the starting materials for the biooxidation. The two structurally complex substrates (**141** and **142**) were not commercially available. Suzuki coupling of phenyl borate and the corresponding

polysubstituted aromatic bromide rendered the oxygenated biphenyls in excellent yield. The preparation of the required aromatic bromides **148** and **152** was synthetically laborious (Figures 45 and 46). Both compounds were known but neither was commercially available. 2,3-Dimethoxy benzene (**148**) was prepared following a specific procedure for the ortho bromination of phenols (Figure 45).[109]



Conditions: (a) Br_2, dibutylamine, toluene, -78 °C, 50-60%. (b) MeI, Na_2CO_3, acetone, r.t., 90%. (c) phenylborate, $Pd(PPh_3)_4$, aq. Na_2CO_3, benzene/ethanol (10/1), reflux, 80%.

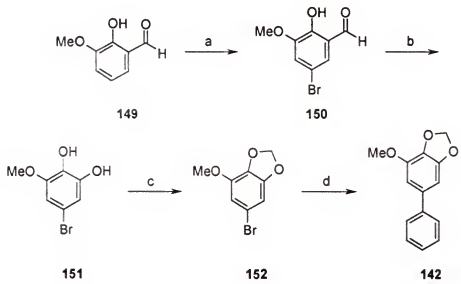
Figure 45. Preparation of substrate **141**.

This methodology has been successfully applied by Hoshino[49] in his synthesis of lycoramine and by Hudlicky in a recent approach to morphine. The procedure relies on the initial formation of *N*-bromodibutylamine by reaction of the amine and bromine at low temperature. Then, guaiacol (**146**) was added to the *N*-bromo compound which was the actual brominating agent. Other procedures reported in the literature such as the use of *N*-bromosuccinimide or bromination in the presence of silver nitrate were not

successful. The *ortho*-bromoguaicol (**147**) was obtained as an 8:1 mixture with the *para*-substituted isomer, along with unreacted guaiacol. The compound was purified by repeated distillation through a short path vigreaux column at reduced pressure until a fraction containing 95% (GC) of **147** was collected. The isolated yield of the compound was never above 60%. At this point the compound crystallized in the collection flask and was stored under argon at 0°C, avoiding exposure to light. Previous experience in the Hudlicky group indicated that the compound was unstable but we found that it can be stored indefinitely if the above precautions were taken. The bromoguaicol was then methylated with methyl iodide in acetone using sodium carbonate as a base to render ether **148** in 90% yield. Originally, the Stille coupling[110,111] was tested for the preparation of biphenyl **141**, but this reaction was promptly abandoned and substituted for the technically easier, and environmentally more friendly Suzuki coupling.[112,113] The latter also provided higher yields and is potentially employable in the presence of a much broader collection of functional groups. The coupling with phenyl borate was performed under standard Suzuki-Miyaura conditions.[112] The reaction was carried out in a benzene-water-ethanol mixture at reflux, using two equivalents of aqueous sodium carbonate as the base and 0.03 equivalents of palladium tetrakistriphenylphosphine as the catalyst. The coupled product was obtained in 80% isolated yield together with 15% of biphenyl (**136**) (Figure 45), a frequently observed byproduct in this reaction.[114] The success achieved in this simple reaction prompted us to explore the Suzuki coupling of more sophisticated molecules (see following sections). Unfortunately, the high yield obtained in the Suzuki did not counterbalance the poor results obtained in the preparation of bromide **147**. Overall, the yield in the preparation of **141** was slightly above 40%.

5-Bromo-1-methoxy-2,3-benzodioxol (**152**) was synthesized in three steps from *ortho*-vanillin (**149**) utilizing a modification of the known procedure.[115,116] The readily available starting material was first brominated with dibromohydantoin (DBH) to render exclusively the derivative **150**. This reaction was repeated several times and in no situation was any trace of another isomer detected. In the original procedure by Dallacker[115] bromine was used as the brominating reagent. We chose DBH because it gave equivalent or superior results than bromine while it was safer and more convenient to handle. Phenol **150** was treated with hydrogen peroxide under aqueous basic conditions to effect a Baeyer-Villiger oxidation of the carbaldehyde with concomitant hydrolysis of the formate product. The resulting catechol **151** was unstable and difficult to obtain in a pure state. Fortunately, the last step (protection of the catechol as a methylenedioxy group) could be carried out without further purification, albeit in poor yield. Optimization of the reaction conditions improved the results but the final yield of benzodioxol **152** was only fair (from 30 to 40%). Two different solvents, dimethylformamide (DMF) and acetone, and various different reaction temperatures and times were asessed in an attempt to improve this last step. The best conditions found were heating at 80 °C for two to three hours in DMF. The use of acetone as a solvent gave lower yield and even at room temperature generated aldol-type byproducts that complicated the final separation. Either chromatography or recrystallization from hexanes provided the final product. In its pure state, **152** is a white crystalline compound that can be stored at room temperature for months without any appreciable decomposition. Slightly impure product has the form of a dark gray amorphous solid; it is also stable and perfectly amenable for use in the coupling experiments. Suzuki

coupling again worked excellently and the same conditions utilized for the preparation of **141** afforded **142** in 70% isolated yield. Because of the poor effectiveness of the previous step, the overall yield fell below 20%. Fortunately, the sequence consisted of simple steps amenable to scale-up so several grams of product were eventually obtained.



Conditions: (a) DBH, $CHCl_3$, r.t. 8 h. 90%. (b) H_2O_2, NaOH, rt 6 h., 65%. (c) CH_2Br_2, DMF, 80 °C; 2 h, 40%. (d) phenylborate, $Pd(PPh_3)_4$, aq. Na_2CO_3, benzene/ethanol (10/1), reflux, 70%.

Figure 46. Preparation of trioxygenated biphenyl **142**.

With the three oxygenated biphenyls in hand we were ready to evaluate the ability of *E. coli* JM109 (pDTG601A) to hydroxylate these types of substrates. The enzyme was first tested against biphenyl (**136**) that is the natural substrate of biphenyl dioxygenase. The auspicious results obtained suggested that the study of the more substituted biphenyls **140-142** under the same conditions (Figure 44) should prove useful. The optimized results for the three substrates are presented in Table 8.

Table 8. Results in the biotransformation of various oxygenated biphenyls.

Entry	Substrate	Diol	Yield (g/l)	Related alkaloid targets
a	136	137	3.0	model
b	140	143	2.5	morphinans
c	141	144	0.8	morphinans
d	142	145	0.0	phenanthridone

Entries a and b show definitely good results. A yield of 2.5 – 3.0 g/L translated into a production of 20 – 25 grams of diol per run. These types of substrates provided plenty of material to both confirm the absolute stereochemistry of the metabolites and utilize them in synthetic projects (see corresponding sections). Entry c is somewhat disappointing but the amount of diol obtained was enough for some stereochemical studies. For further synthetic work a modified coupling approach was developed. Especially discouraging was the result described in entry d in light of the fatiguing procedure used for preparing the starting material. Fortunately, the key intermediate bromide **152** and the experience gained in the Suzuki coupling were of key value in the synthesis of narciclasine (vide infra).

From these results, it seemed clear that there is a limit in the degree of substitution of the oxygenated ring that the enzyme can tolerate. The key diols **144** and **145** should be prepared by alternative routes in order to provide substantial amounts of material to be used in the synthesis of natural products.

In order to confirm the absolute stereochemistry beyond doubt and simultaneously explore alternative modes of synthesis, it was decided to prepare diols **143** and **144** (or a

simple derivative of these compounds) from an independent and well established source of chirality. 3-Iodo-3,4-cyclohene-1,2-diol (**153**) and its bromo analog are extensively studied metabolites whose absolute stereochemistry is known. These compounds, and their protected derivatives, have been used in the palladium mediated coupling with alkynes[117] and organostannane compounds[108]. The latter example seemed particularly attractive since the authors reported the preparation of a biphenyl derivative by this method. Although successful coupling are reported for diols that preserve the reactive diene functionality it was a more secure bet to work with the partially hydrogenated derivatives that do not undergo acid catalyzed aromatization. In this way, it was decided to transform the new metabolites **143** and **144** into stable derivatives (**154** and **155**) which can also be prepared from iododiol (**153**) in a convergent fashion (Scheme 6).

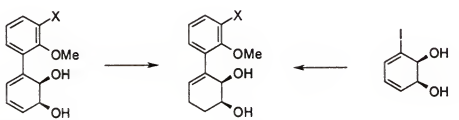


Figure 47. Strategy for confirming the stereochemistry of the new metabolites.

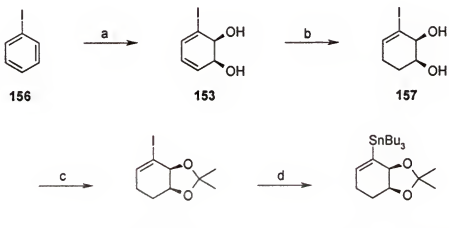
Preparing diols **154** and **155** from the new metabolites was straightforward since it is well established that diimide reduction of cyclohexadiene diols reduces only the less substituted double bond with complete regioselectivity. Diimide was generated by decomposition of potassium azodicarboxylate (PAD) under mildly acidic conditions

according to well detailed procedures.[118] On the other hand, to prepare the reduced diols from diol **153** required a number of steps (Figure 48). Iodobenzene (**156**) was biooxidized by toluene dioxygenase (expressed in *E. coli*) to produce **153** in a yield of 10 g per liter of cell-free medium. The unstable metabolite was reduced with PAD, in the same way and yield (90%) as **143** and **144**, to afford the reduced analog **157** (Figure 49). The latter was protected as the acetonide (**158**) since the diol functional group was incompatible with the conditions for the formation of the organostannane. Acetonide **158** was treated with *sec*-butyllithium at low temperature to effect metal-halogen exchange and immediately transmetalated with tributyltin chloride to render the coupling precursor **159**. Stannane **159** was purified by column chromatography to deliver a product of sufficient purity for the coupling experiments in an overall yield of 50% from **153** (Figure 48).

Having prepared sufficient amounts of **159** it was coupled to either aromatic bromide **148** (prepared as detailed above) or the commercially available **36**. In both cases the yield of the reaction was low probably in view of the known poor performance of electron rich bromides (particularly methoxy substituted bromides) in the Stille coupling.[119,120] The coupled acetonides **161** and **162** were deprotected utilizing an acidic resin in a methanol-water mixture to afford diols **154** and **155** that matched the spectroscopic data of the compounds prepared from the new metabolites.

The absolute stereochemistry of diols **143** and **144** was unequivocally proven by comparison of the optical rotation of the reduced derivatives **154** and **155** respectively to the corresponding value obtained for the same compounds prepared from the known diol

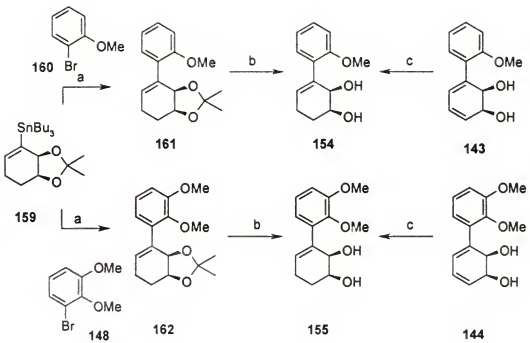
153 (Figure 49). Since the values obtained in either case were identical (within experimental error) the stereochemistry of **143** and **144** was assigned as shown.



Conditions: (a) *E. coli* JM109 (pDTG601A), 10 g/L. (b) PAD, HOAc, MeOH, 0 °C – r.t., 12 h., 90%. (c) DMP, acetone, r.t., 30 m. 95%. (d) *s*-BuLi, MeOH, -78 °C, 15 m., then $ClSnBu_3$, 2 h., 60%.

Figure 48. Preparation of the optically pure organostannane derivative **159** from iododiol **153**.

In summary, two new metabolites from the microbial oxidation of oxygen-containing biphenyls were isolated and identified. The resulting diols were obtained as the sole product of the biotransformation and constitute the first example of this class of metabolites isolated from oxygenated biphenyls. The monosubstitution of one aromatic ring did not affect the ability of the enzyme to oxidize the other (unsubstituted) ring. On the other hand, disubstitution significantly decreased the viability of biphenyls as substrates while trisubstituted compounds could not be metabolized.



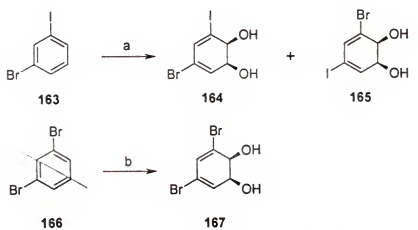
Conditions: (a) $Pd(PPh_3)_4$, LiCl, toluene, 110 °C, 24 h. 30%. (b) Amberlyst 15 (wet), MeOH-H_2O (10/1), 45°C, 80%. (c) PAD, HOAc, MeOH, 0 °C to r.t., 12 h., 90%.

Figure 49. Preparation of diols **154** and **155** by two independent routes.

3.1.3. Enzymatic Dihydroxylation of *meta*-Substituted Halobenzenes

As a part of a synthetic effort towards the total synthesis of narciclasine (vide infra) it became relevant to prepare a 3,5-substituted halocyclohexadiene diol of type **164**, **165** or **167** (Figure 50). We were interested in a diol that could potentially form a bond between C5 (narciclasine numbering) and an aromatic ring. As was mentioned before 3,5-substituted diols are optically pure but might produce a mixture of regioisomers if the enzyme cannot absolutely differentiate between the two substituents. On the other hand, a *meta*-substituted aromatic ring bearing two identical substituents must provide on biooxidation a single regioisomer of high enantioputity by virtue of its diagonal plane of symmetry.

Literature data on 3,5-disubstituted diols suggested that the corresponding precursors were not easily metabolized. The examples were scarce and the yields reported are low.[91,121] Since 3-iodo-5-bromo cyclohexadiene diol was known,[121] that biooxidation was repeated in order to check *E. coli* JM109 (pDTG601A) in the reaction and to compare the yield obtained with the previously reported. Although the biooxidation of substituted benzene **163** must render two diols (**164** and **165**), metabolite **164** should be



Conditions: (a) *Pseudomonas putida* UV4 or *Escherichia coli* JM109 (pDTG601A), 0.5 g/L. (b) *Escherichia coli* JM109 (pDTG601A), 3-4 g/L.

Figure 50. Biooxidation of *meta*-disubstituted benzenes.

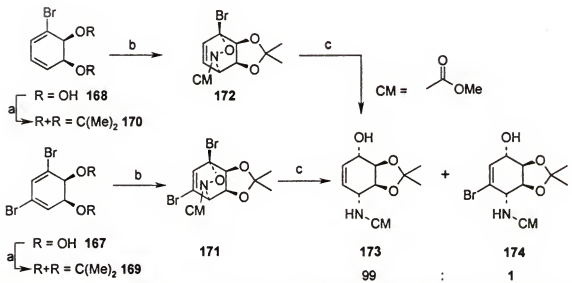
the major one and for synthetic purposes it would be possible to proceed in the synthesis even with the unseparated mixture of isomers. Unfortunately, only 4 grams of diol were isolated from the biooxidation. This represented a yield of 0.5 grams per liter, which fell under the limit of the synthetically useful for these kinds of procedures. In addition, the diols and their derivatives proved to be very unstable and prone to quick decomposition with loss of iodine. These results encouraged the trial of the biooxidation of *meta*-

dibromobenzene (**166**), which was not previously reported, as a way to obtain a more suitable material for synthetic work and at the same time study the optical purity of the biooxidation in a symmetric *meta*-disubstituted benzene. Surprisingly, diol **167** was obtained in a very good yield (3-4 g/L) and it was a relatively stable compound that could be stored indefinitely at low temperature (-78 °C).

A possible way to confirm the absolute stereochemistry of this metabolite was to convert it into the known conduramine A derivative **173** that is also prepared from the thoroughly studied 3-bromocyclohexadiene diol **168** (Figure 51). The same sequence of reactions was applied to both metabolites. First, they were protected as the corresponding acetonides (**169** and **170**) in high yield. This was followed by Diels-Alder reaction with a acylnitrosyl dienophile generated *in situ* from the corresponding hydroxamic acid that led to adducts **171** and **172** as the only products. Reductive cleavage of the N-O bond in the oxazines with aluminum amalgam occurred with attendant dehalogenation as previously reported.[35] This act preserved the *syn*-relationship of the hydroxyl and the protected amino groups established during the cycloaddition. In the case of the dibrominated adduct **171** the aluminum amalgam also removed the vinylic bromine leading almost exclusively to alcohol **173**. Since the optical rotation values found for **173** obtained from either diol precursor are identical (within experimental error), and since the absolute stereochemistry of bromo diol **168** is well established,[86,122,123] we could establish the absolute stereochemistry of the new metabolite **167** to be as shown (*1S, 2S*). The concomitant debromination of the vinylic bromine atom at C5 that was convenient at this point became a problem for the synthetic purpose pursued later. Different strategies to

obtain amino alcohol **174** using distinct reduction techniques needed to be developed to overcome this problem.

In summary, we have prepared four new metabolites of toluene dioxygenase degradation of aromatics and determined their absolute stereochemistry. All four compounds presented particular structures that challenged substrate specificity of the enzyme but enhanced the synthetic potential of the diols. In the process of structure elucidation, we also found that the palladium coupling might be a viable alternative for the preparation of these type of compounds whenever the yield of biooxidation was unacceptably low. Three of these compounds (diols **143**, **144**, and **167**) were of immediate use in our synthetic projects. The results obtained in those ventures are described in the following sections.



Conditions: (a) DMP, acetone, r.t., 0.5 h. (b) $MeCO_2NHOH$, Bu_4NIO_4, CH_2Cl_2, r.t., 12 h. (c) Al(Hg), THF-H_2O, 0°C to r.t., 4 h.

Figure 51. Preparation of a conduramine derivative from diol **43** to confirm its absolute stereochemistry.

3.2. Chemoenzymatic Synthesis of Unnatural Amino Acids. Approach to Morphine Synthesis

Of the five stereogenic centers in morphine (**1**), the most difficult to control in a relative sense are C9 and C14.[74] A possible disconnection of the morphine skeleton, not yet reduced to practice, indicates that the target molecule can be derived from a suitably functionalized β-cyclohexenyl amino acid such as **175**, obtained via a [3.3]-sigmatropic rearrangement of a substituted glycine ester enolate derived from **176**, in which the chirality is set by arene dioxygenase oxidation of an aromatic precursor (Figure 52).

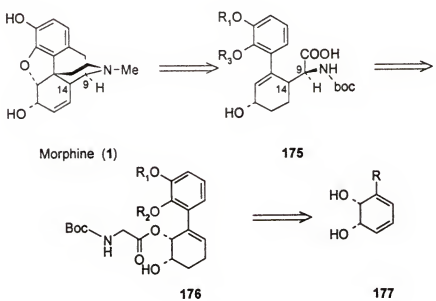


Figure 52. A disconnection or morphine that sets the stereochemistry of C-9 and C-14 at an early stage of the synthesis.

As an added benefit, this strategy allowed us to prepare a series of β-substituted amino acids bearing chiral side chains of moderate complexity in a few steps. The experience gained in the preparation of biphenyl diols by either direct biooxidation or by palladium coupling (see previous section) served as a starting point to explore this

approach. The application of the Claisen rearrangement to transfer stereochemical information within a *cis*-cyclohexadiene diol molecule has not been reported before but it was suggested in the first publication in the area by Hudlicky *et al.*[124] In this approach we decided to perform the rearrangement of amino esters **176** following the protocol of Kazmaier that is a remarkable improvement from the previously reported methods for Claisen rearrangement of similar substrates (see Chapter 2: Historical Background).

3.2.1. Preparation of the Rearrangement Precursors

A series of five amino esters of type **9** were prepared following standard procedures (Figure 54). The key step in this preparation was the chemoenzymatic synthesis of the chiral diols **182**, **183**, **137**, **143**, and **144** in an optical pure fashion. The simple diols **182** and **183** were obtained from the enzymatic oxidation of toluene (**180**) and chlorobenzene (**181**) respectively. These compounds are excellent substrates for toluene dioxygenase. In fact, toluene is the natural substrate of the enzyme and its product of oxidation (diol **182**) was the second metabolite whose stereochemistry was proved beyond doubt.[125] Chlorobenzene is also an excellent substrate[86,122] and one of the few that can induce the production of toluene dioxygenase by pseudomonas species.[86] This means that no previous induction with toluene is required to initiate the transcription of the enzyme and therefore indicates that chlorobenzene cannot be distinguished from toluene by the bacteria. Both the cyclohexadiene-*cis*-diol derived from toluene and chlorobenzene were the firsts of the class of metabolites to be exploited in organic synthesis.[1,124,126] The oxidation of these two compound can be carried out by either the mutant strain *Pseudomonas putida* F39/D[8] or by the more potent recombinant organism *Escherichia coli* JM109(pDTG601A).[12] For practical reasons, we chose to utilize the

pseudomonas strain for toluene and the *E. coli* for chlorobenzene. The oxidation of toluene, carried out in a two liter fermenter, yielded seven grams of (+)-*cis*-1,2-dihydroxy-3-methylcyclohexa-3,5-diene (**182**) that was enough for our synthetic purposes. (+)-*Cis*-1,2-dihydroxy-3-chlorocyclohexa-3,5-diene (**183**) was prepared in a twelve liter fermenter (the actual working volume is 10 liters) and 80 grams were recovered from the broth. The preparation of the corresponding diols from biphenyls **136**, **140**, and **141** was not customary biooxidation work and was described in the previous section.

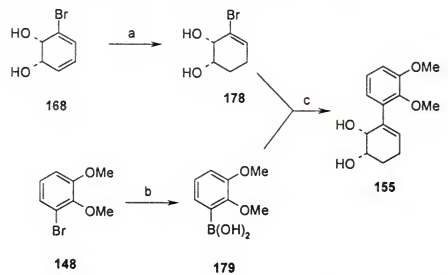
As it was mentioned in section 3.1.2. biphenyl **141** was not a good substrate for the enzyme and the corresponding diol **144** was obtained in very low yield. This result prompted us to abandon the direct biooxidation of **141** and look for an alternative route (Figure 53). From our previous work with the metabolites (vide supra) we knew that it was feasible to couple a vinylic stannane derived from a diol to an aromatic bromide (Figure 49). In that study we observed that the Stille conditions resulted in yields never above 35% so we turned to the Suzuki reaction. The Suzuki coupling has become one of the most effective methods for carbon-carbon bond formation between an aromatic ring and a Sp2 center.[113] Originally, the methodology was limited to iodides and some reactive bromides but it has expanded to triflates[127] and more recently to chlorides.[128-131] A wide variety of catalysts and conditions are reported in the literature. The information available is not always complementary and often contradictory. For example, Pd(0) catalysts are the most popular but the more stable Pd(II) catalysts are frequently mentioned.[132] An important merit of the Suzuki coupling is the broad tolerance of functional groups that can be present in the coupling partners because of the mild

conditions of the reaction. The typical temperature for the reaction is the refluxing temperature of a heterogeneous mixture of low or medium boiling point solvents. Representative mixtures are benzene-H_2O, tetrahydrofuran-H_2O, toluene-H_2O, and benzene-H_2O-ethanol. Nevertheless more sophisticated solvents are sometimes reported, such as 1-methyl-2-pyrrolidone that is claimed to be especially suitable for the coupling with aryl chlorides.[131] In addition some reports on the Suzuki coupling of biaryls at room temperature have appear on the literature.[133,134] These procedures reported newer more active catalysts or just different bases. For example, the procedure of Anderson[133] for ambient temperature coupling is basically identical to the standard Suzuki-Miyaura conditions[112] but uses thallium hydroxide instead of sodium carbonate as a base. The reaction continues to improve and progress to milder and environmentally friendlier conditions. In a remarkable recent report, the use of "ligandless" palladium catalysts in aqueous media has been described.[135]

The especially mild conditions of the coupling allowed us to couple bromide **178** and borate **179** (prepared from bromide **148**) without protection of the diol. A possible problem was the low solubility of **178** in benzene, but it completely dissolved in the refluxing mixture of benzene-water and ethanol used in the reaction. Although several newly reported conditions were tried, the typical procedure of Miyaura and Suzuki:[112] benzene as the main solvent, $Pd(PPh_3)_4$ as catalyst and a two molar excess of aqueous sodium carbonate as the base provided the best results in our hands for the preparation of diol **155** (Figure 53). To date, this is the only example of Suzuki coupling of an unprotected *cis*-diol and an aromatic borate. This work complemented the work of

Hudlicky and Boros in palladium catalyzed coupling of acetylenes with free diols[117] as well as the reports from Ley *et al.*[108] and Boyd *et al.*[123] on Stille-type couplings.

In this way, either by direct biooxidation or by a combination of biooxidation of a simpler substrate, diimide reduction, and Suzuki coupling we were able to obtain reasonable amounts of diols **184-186**, **154**, and **155** for synthetic purposes. The distal OH was protected as a thexyldimethylsilyl ether by reaction with thexyldimethylsilyl chloride. This reagent is commonly abbreviated as THSCl but its proper acronym is TDSCl[136]. TDS ethers are slightly more stable and hindered than TBDMS ethers. On the other hand



Conditions: (a) PAD, HOAc, MeOH, 0 °C – r.t., 12 h., 95%. (b) *t*-BuLi, THF, -78°C, then $B(OEt)_3$ or Mg, THF, reflux, then B(OEt)3, 70-80%. (c) 0.03% eq. $Pd(PPh_3)_4$, aq. Na_2CO_3, PhH-EtOH, reflux, 3h. 65-85%.

Figure 53. Preparation of enantiomerically pure 2,3-dimethoxybiphenyl diol by Suzuki coupling.

TDSCl is less expensive and easier to handle (its a liquid) than TBDMSCl. It is well known in the Hudlicky group[137] that TDSCl is distinctively selective for protecting only the distal hydroxyl group in these type of arene metabolites. The protected derivatives

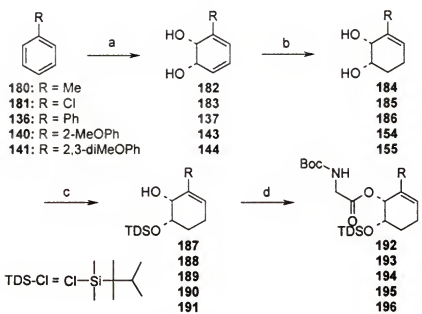
187-191 were coupled with Boc-glycine under standard DCC conditions[138]. After protection of the distal hydroxyl group as a TDS ether the proximal hydroxyl is considerably hindered and shows a poor reactivity. As a matter of fact, even simple acetylation under standard conditions (acetic anhydride, DMAP in pyridine) is only completed after 24 hours. The DCC coupling was not an exception and even with a four molar-equivalents excess of amino acid and DCC the reactions times were long. Particularly, for the aromatic derivatives **189-191** that presented extra hindrance around the allylic alcohol the starting material was consumed only after 48 hours. Careful chromatography to separate the products from the dicyclohexylurea, allowed isolation of the products in good yields. Attempts to substitute the DCC for EDC, a popular carbodiimide among the peptide chemists, that renders a soluble urea as the byproduct did not reproduce the good yields obtained with DCC.

3.2.2. Claisen Rearrangement Studies

The first exploratory studies to find conditions for the Claisen rearrangement were performed on amino ester **194** (R = Ph) which was easily available to us and could be used as a model for the more sophisticated dimethoxy derivative **196**. Our first choice was to test the standard ester enolate Claisen rearrangement protocol.[58] In our hands, these conditions failed to provide any rearranged products either with or without trapping the enolate as a silyl ether. After several attempts, we turned to the Kazmaier chelated-enolate Claisen rearrangement that yielded rearranged products **197** and **198** in 80 % overall yield. Noticeably, an attempt to perform the rearrangement on the *N*-methyl derivative of **194** (obtained by DCC coupling of alcohol **189** and sarcosine) resulted only in high yield recovery of the starting material. This result, added to the previous reports

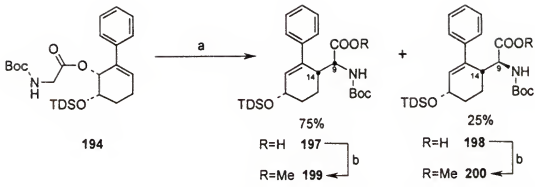
from Bartlett and Kazmaier[60,61] indicated that chelation is probably a prerequisite for the rearrangement to occur.

Amino acids **197** and **198** were isolated as sticky oils that solidify at 4 °C after several days as amorphous solids. They were difficult to chromatograph and virtually impossible to obtain in the pure state by standard column chromatography.[139] Several conditions of solvent mixtures and flow rates were tried using a C18 column but HPLC did not provide an efficient separation. We then decided to derivatize the amino acids *in situ* as the corresponding methyl esters **199** and **200** that were easily separable. The two



Conditions: (a) toluene dioxygenase expressed in *Pseudomonas putida* F39/D (R = Me (3.5 g/L)) or *Escherichia coli* JM109 (pDTG601A) (R = Cl (10.0 g/L), Ph (3.0 g/L), 2-MeOPh (2.5 g/L), 2,3-diMeOPh (0.8 g/L). (b) PAD, HOAc, MeOH, 0 °C – rt, 12 h., 85-95%. (c) TDSCl, imidazol, DMF, -5 °C, 8 h., 80-90%. (d) Boc-Gly, DCC, DMAP, CH_2Cl_2, r.t., 24-48 h., 75-90%.

Figure 54. Preparation of amino esters precursors for the chelate-enolate Claisen rearrangement.



Conditions: (a) LDA (2.2 eq), anhyd. $ZnCl_2$ (1.1 eq.), THF, -78 °C to r.t., 12 h., 80%. (b) CH_2N_2, Et_2O, r.t. 5 min.

Figure 54. Chelated-enolate Claisen rearrangement of biphenyl amino ester **194**. (The ratio of **197/198** was estimated from the integration of isolated signals in the ^1H NMR spectrum of the mixture -uncertainty: ± 5%-).

compounds presented very similar spectroscopic properties that immediately identified them as the two expected epimers. Their respective proton nuclear magnetic resonance (^1H NMR) spectrum were almost identical and presented a non-conclusive splitting pattern for the signals corresponding to the protons at the key stereocenters C9 and C14 (see Appendix: Selected Spectra). The signals were broad and poorly resolved probably because of the restricted rotation around the C9-C14 bond and the tautomerism of the carbamate group. Interestingly, the respective signals corresponding to the C14 protons (H_a) showed a very different chemical shift. The compound that was later identified as amino ester **199** presented a broad singlet at 4.27 ppm that was assigned to H_a. The epimer **200** presented a similar signal at 3.32 ppm that also was assigned to H_a. The rationalization of this shift is not clear, especially because C14 is not the epimeric center. However, this shifting that was observed also in the free amino acids, was an important factor for the assignment. It was not possible to infer further stereochemical data from

the methyl esters and we realized that it would be necessary to transform the products of the rearrangement into more rigid structures. We anticipated that a rigid backbone should provide better and more complete information to identify the major isomer obtained in the rearrangement.

An immediate possibility to attain a rigid system was to remove the TDS protecting group and transform the crude mixture of amino acid **197** and **198** into the corresponding lactones. Therefore, the amino acids were treated with recrystallized toluensulphonic acid in anhydrous dichloromethane at room temperature and the TDS group was cleaved. Contiguously to the deprotection of the alcohol rapid formation of lactone **203** was observed (Figure 55). In principle, two possible lactones (**202** and **203**) could have formed derived from the two resonance forms of cation **201**. We rationalize the results in the greater stability of resonance form **201b** that should contribute significantly to the hybrid. Moreover the competition between formation of a five over a seven membered ring favors the former from both kinetic and thermodynamic considerations.

Lactone **203** derived from the major product of the rearrangement was isolated as a crystalline white solid. The presence of the five-membered ring lactone was indicated by signals in the infrared (IR) spectrum at 1773 and 1161 cm^{-1}. In addition, the ^{13}C NMR spectrum showed a signal at 174.4 ppm characteristic of esters and lactones. The migration of the double bond out of the conjugation was suggested by the low extinction coefficient at 254 nm compared to the parent compound. The bottleneck of the structure elucidation was to determine the *cis* or *trans* relationship between protons H_a and H_b on carbons C9 and C14 respectively. Lactone **203** showed a coupling constant of 7 Hz for

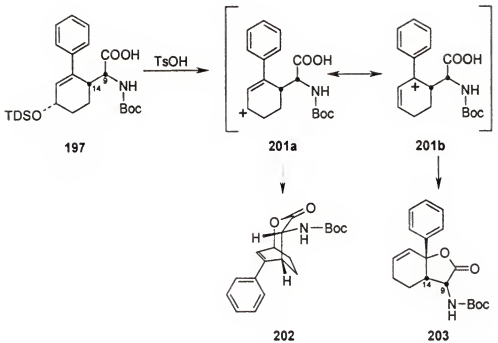


Figure 55. Lactonization of amino acid **197** (lactone **202** was never observed)

protons H_a and H_b. This value corresponds to a dihedral angle of 20° according to the Karplus equation. Unfortunately, none of these two signals was well resolved in the corresponding epimeric lactone (**204**) derived from the minor isomer. In fact, the chemical shift of the signal assigned to H_a was displaced upfield withrespect to the major lactone **203** as in the free amino acids and in the methyl esters. In the spectrum of lactone **204** H_a was displaced so far upfield that it overlapped with the methylene signals of the six membered ring, making the analysis impossible. Since considerable amounts of pure lactone **203** were available, we were able to perform an Heteronuclear Correlation (HETCOR) experiment and fully assign the proton and carbon signals. With that data in hand, we attempted to obtain spatial correlation information from a Nuclear Overhausser Effect (NOE) experiment. This experiment was successful for lactone **203** (Figure 56).

The NOE experiment showed a positive correlation (measured with the digital integrator) between the signal assigned to H_b and the broad singlet assigned to the protons in the aromatic ring. We could also measure a positive correlation between the signal assigned to the proton in the amino group and the α-methylene proton on C8 (morphine numbering).

The NOE experiment strongly indicated that the structure of lactone **203** contained a *cis* relationship between protons Ha and Hb. However, it is not desirable to rely only in NOE information (particularly if good data is not available for both isomers). Since we managed to obtain fifty milligrams of pure lactone **203**, we attempted to obtain a good quality crystal by slow evaporation of an appropriate solvent. After only a few assays benzene provided a single crystal of **203** that was subjected to X-ray analysis. The resulting crystallographic data confirmed the structure suggested from the ^{1}H NMR data is shown in Figure 57. The *cis* relationship between H_a and H_b is clearly shown. The dihedral angle between H_a and H_b was 23° in the crystal; this is in good agreement with coupling constant of 7 hertz that was observed in the ^{1}H NMR spectrum. Another noticeable aspect resulting from the diagram is that it shows the clear concavity of the six-five fused system. The location of the bulky Boc group on the endo face of the molecule suggested the possibility of epimerization of lactone **203** to the thermodynamically more stable **204** that positions the Boc group in the exo face of the molecule.

With this result in hand for the rearrangement of amino ester **194**, it was decided to compare it with the results attainable for the rest of the amino esters in the series. Specifically, we were interested in the result for the synthetically relevant dimethoxy

phenyl derivative **196**. Therefore, the other four members of the series (see Figure 54) were subjected to same Kazmaier conditions. The results obtained are depicted in Figure 58 and Table 9.

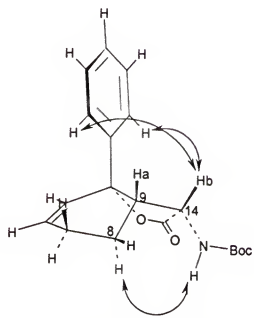


Figure 56. Relevant positive NOE correlations in lactone **203**.

The ratio of the rearranged amino acids, epimeric at C-9 (morphine numbering), was determined by ^{1}H-NMR analysis of the crude mixtures. The relative and absolute stereochemistry of all amino acids was determined by transforming them to the corresponding lactone and or methyl ester derivatives (Figure 58). Since the structure of lactone **203** was unambiguously established by X-ray structural analysis, the absolute stereochemistry of the amino acid **197** was assigned as shown. Comparison of spectral data of the rest of the amino acids and their corresponding derivatives established their stereochemistry. In summary, the assigned stereochemistry of the major amino acids is 2*R*,3*R* (2*R*,3*S* in the case of the chloro compound **206**) and 2*S*,3*R* respectively.

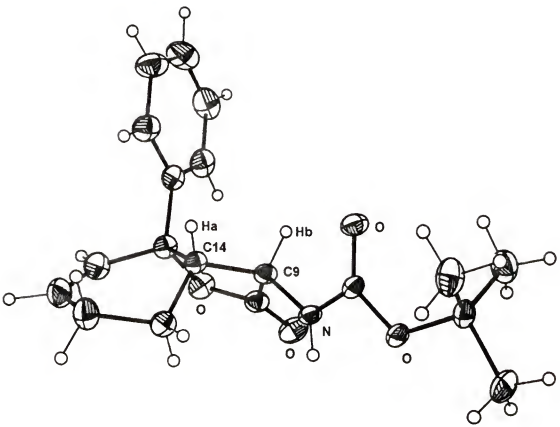
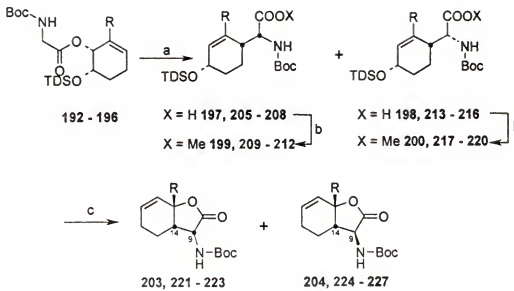


Figure 57. Crystal structure of lactone **203**.



Notes: The lactones of the chloro series were not prepared.
The methyl esters of the dimethoxyphenyl series were unseparable by column chromatography.

Conditions: (a) LDA (2.4 eq.), $ZnCl_2$ (1.2 eq.), THF, -78 °C to r.t. (b) MeCN, Et_2O, r.t. 5 min. (c) TsOH, CH_2Cl_2, r.t. 2-6 h.

Figure 58. Summary of the results in the rearrangement of amino esters **192 - 196**. See also Table 9.

Table 9. Summary of results in the chelated-enolate Claisen rearrangement of amino esters **192 - 196**.

entry	R	substrate	major product	methyl ester	lactone	yield (%)	*cis* (%)	*trans* (%)
1	Me	192	205	209	221	80	75	25
2	Cl	193	206	210	-	15	90	10
3	Ph	194	197	199	203	80	75	25
4	2-MeOPh	195	207	211	222	75	75	25
5	2,3-DiMeOPh	196	208	212	223	65	65	35

These results are somewhat surprising since the configuration at the α-amino position for the major product is reversed from that reported for the closely related cycloalkenyl amino esters reported by Kazmaier[140] (see Chapter 2, Table 6). Because of the fixed enolate geometry arising from chelate formation, the stereochemical outcome of the rearrangement depends exclusively on the preference for either a chair or a boat transition state (see Chapter 2). The results are rationalized in terms of the rearrangement proceeding via a chair-like transition state. For six-membered ring substrates the preference for a boat-like transition state is generally accepted.[62,141,142] This is based on the presence of steric interactions in a chair-like transition state between the cyclohexenyl ring and the solvated metal, which are absent in a boat-like transition state (Figure 59). As Ireland and coworkers have pointed out for the rearrangement of the related silylketene acetals,[62] with cyclohexene derivatives both chair and boat transition states should be expected, depending on the size and position of the substituents on the ring. The effect of the bulky silyl ether may be considered negligible, as evidenced by results of these rearrangements reported in the carbohydrate field in which no changes in selectivity were reported with bulky oxygenated substituents on the ring.[143] Conversely, the cyclohexenyl derivatives used in this study bear substituents at the α-position of the allylic carbon. These substituents may interact unfavorably with the solvated metal in a boat transition state as shown in Figure 59. As a result of the two opposing steric interactions, the cyclohexenyl ring destabilizes a chair transition state, and the substituents on the α-position of the allylic carbon destabilize a boat transition state. Consequently, the energy difference between both transition states is small. This is in

accord with observed product selectivities ranging from 2:1 to 9:1 ratio for the series (Table 9) with a chair transition state always predominating.

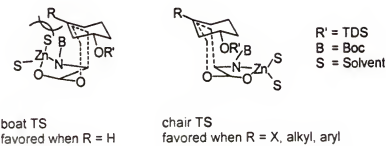


Figure 59. Two possible transition states in the rearrangement of amino esters **192-196**.

The observed selectivity could also be explained by proposing that no or incomplete chelation is taking place and therefore the combination of a boat-like transition state and a *trans*-enolate is dictating the major product (Figure 60). However, the conclusion of Bartlett on the generally weakly chelated lithium enolates was that chelation is strong in this type of systems and that a five membered ring chelate formed in the reaction before the enolate trapping.[57] In addition, Kazmaier for a series of metal-chelated enolates concluded: "In contrast with the corresponding lithium enolates which do not show this rearrangement because they decompose during warming, the chelate-enolates are much more stable."[61] These conclusions agree with the result obtained in the case of the *N*-methyl derivative of **194** that failed to rearrange in several attempts. On the other hand, the hypothesis of the partial or incomplete chelation is attractive since it explains better the decrease in selectivity for the bulkiest member of the series (entry 5 in Table 9). The presence of additional Lewis bases in the molecule could offer alternatives

sites of chelation to the zinc cation allowing the formation of a free enolate. This possibility was tested on amino ester **196** by adding a three-fold excess of $ZnCl_2$ to the reaction mixture but no improvements of selectivity were observed.

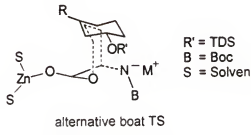


Figure 60. A non-chelated boat-like transition state could dictate the stereochemistry of the products.

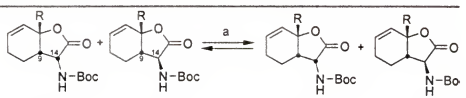
The lack of stereoselectivity drew our attention to the possibility of epimerization of the lactones to their minor isomers, since the bulky protected amino group is situated on the concave face of the bicyclic system. Accordingly, **203** epimerized to the more stable **204** (80% after 37 hours) when treated with DBU in THF at room temperature and **222** epimerized to the corresponding **225** albeit to a lesser extent (72 % after 72 hours). When the crude mixture of rearranged amino acids was subjected to lactonization conditions, the resulting mixture of lactones was formed. Treatment of this material with DBU provided the thermodynamically equilibrated products. (Table 10). The epimerized lactones **204** and **205** contain the same relative stereochemistry as morphine at the crucial centers C9 and C14, and are ideally suited for further elaboration.

3.2.3. Conclusion

A chemoenzymatic strategy based on toluene dioxygenase dihydroxylation and Claisen rearrangement was successfully applied to the synthesis of a series of ten chiral

unnatural amino acids. The chelated-enolate Claisen rearrangement, first discovered by Kazmaier *et al.*, was applied to substrates of greater complexity and therefore the scope and limitations of this new reaction have been studied. The effectiveness of the method for the preparation of unnatural amino acids derived from asymmetric diols was demonstrated.

Table 10. Results obtained in the epimerization experiments.



203 **204** **203** **204**
222 **225** **222** **225**

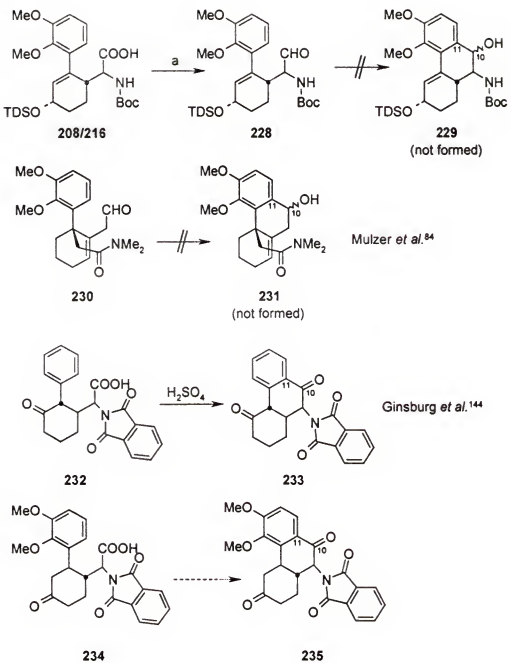
R	HPLC ratio		HPLC ratio	
Ph	75	25	20	80
2-MeOPh	75	25	28	72

The compounds prepared and specifically the dimethoxy derivatives are of potential application in morphine synthesis. The rearrangement set correctly the stereochemistry at C14 (morphine numbering) with complete stereoselectivity. On the other hand, the stereoselectivity at C9 was only fair and the major products obtained showed stereochemistry opposite to the present in natural morphine at that key position. These results suggested that in this case the rearrangement did not go through a boat-like transition state (as it is commonly accepted for cyclic substrates). Another possibility to

explain these results is to recognize that chelation did not occur completely and therefore a *trans*-enolate was involved in the reaction.

In order to convert these results into a true approach to morphine, the closure of the C10-C11 bond (morphine numbering) should be achieved. Preliminary studies on that reaction by a Friedel-Crafts type acylation of aldehydes **228** have resulted in complete decomposition of the starting material or deprotection of the allylic alcohol. No traces of closed product have been detected. This result is in accord with a recent report by Mulzer[84] on the unsuccessful closure of the related system **230** under a variety of conditions (Figure 61). On the other hand, precedent in the classic morphine research of Ginsburg[144] indicates that amino acids of type **232** cyclize in acidic conditions to render diketones **233**. This result strongly suggests that a ketone of type **234**, easily accessible from amino acid **216** (or the corresponding lactone **227**), should cyclize in the desired fashion.

Apparently, the allylic hydroxyl group, the olefin or the Boc protective group are disturbing the C10-C11 closure. Deeper studies on the influence in the Friedel-Crafts closure of the rich functionality present in these systems are necessary in order to reduce to practice this incipient approach to the morphinan skeleton.



Conditions: (a) i. CH_2N_2, ether, r.t.; ii. DIBAL, CH_2Cl_2 –78 °C, 30 min. **65%**

Figure 61. Literature precedence and preliminary results on the C10-C11 closure (morphine numbering).

3.3. Chemoenzymatic Synthesis of Narciclasine

In Chapter 1 of this dissertation the hypothesis that structurally diverse oxygenated alkaloids could be accessed from a few phenyl substituted cyclohexadiene diols derived from bacterial metabolites was proposed. In the previous sections of this Chapter we have discussed the chemoenzymatic syntheses of such diols and their application to the synthesis of unnatural amino acids and to approach the morphinan skeleton. In order to prove the initial statement and to show the scope of the strategy we prepared the bioactive alkaloid narciclasine by a short convergent strategy starting from two simple aromatic pieces (Figure 62).

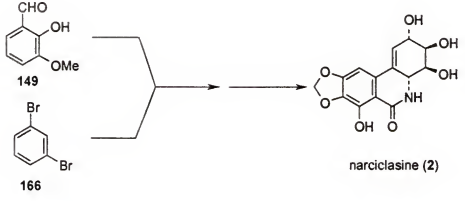
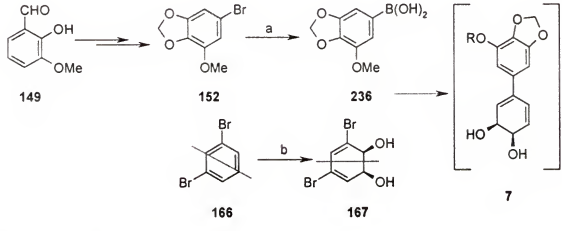


Figure 62. Convergent strategy toward narciclasine from two aromatic building blocs.

In this strategy, dibromobenzene **(166)** provided the asymmetric portion of the molecule by means of toluene dioxygenase oxidation to the corresponding *cis*-cyclohexadiene diol **(167)**. *ortho*-Vanillin **(149)** afforded the aromatic fragment of narciclasine in the form of borate **236** derived from bromide **152** (for a discussion on the synthesis of these fragments see the first section of the chapter). In this fashion, the

coupling of fragments **167** and **236** (Figure 63) could provide a synthetic equivalent of diol **7** introduced in Figure 2. Preparation of the actual diol **7** requires addressing the issue of differentiation between two, chemically almost identical, vinylic bromides. The bromine atoms are located in different proenantiotopic planes by virtue of the particular symmetry present in the *cis*-cyclohexadienediols. This symmetry has been extensively exploited in synthesis by Hudlicky *et al.*[7,43,126,145,146]

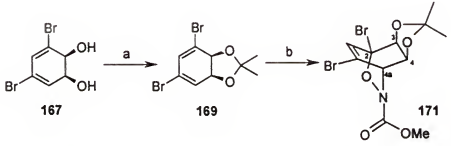


Conditions: (a) *t*-BuLi, THF, -78°C, then $B(OEt)_3$.

Figure 63. Generation of the "biphenyl diol" precursor.

Interestingly, in the highly symmetric dibromobenzene (**166**) the two bromine atoms are identical and cannot be differentiated by any means. However, the enzymatic oxidation degrades this symmetry (or transfers asymmetry from the enzyme to the substrate) and the two bromine atoms become slightly different, although still chemically very similar (Figure 63). It was possible to take advantage of this small difference and modify diol **167** to enhance the chemical discrimination to a point where the selective coupling with the aromatic piece became feasible. This was achieved by first converting

diol **167** into the acetonide derivative **169** that provided an effective blocking of the α-face of the molecule. The acetonide was reacted with the acylnitrosyl dienophile derived from methylcarbamic acid to provide oxazine **171** as the only product (Figure 64). This remarkable transformation was previously mentioned in the first section of the chapter since it was applied to confirm the absolute stereochemistry of diol **167**. There is ample precedence in the reaction of *cis*-halocyclohexadienediols with nitrosyl nucleophiles. This strategy formed the basis of Hudlicky's synthesis of amino cylitols,[42,46,47,147] and in the preparation of amaryllidaceae alkaloid lycoricidine[35,46] More recently the reaction was applied to the synthesis of the unnatural alkaloid *ent*-7-deoxypancratistatin.[100]



Conditions: (a) DMP, acetone, r.t., 0.5 h. (b) $MeCO_2NHOH$, Bu_4NIO_4, CH_2Cl_2, r.t., 12h.
Figure 64. Manipulation of diol **167** to allow the efficient coupling with borate **236**.

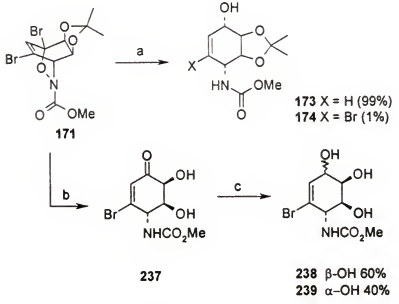
From the previous work of Olivo and Hudlicky mentioned above it is well established that the cycloaddition takes place exclusively to form the isomer where the halogen and the oxygen atoms are attached to the same carbon (*meta*-isomer).[42,46] In accordance with those results, we isolated oxazine **171** as the only product. The structure of **171**, that was suggested by spectroscopic data, was confirmed by transforming **171** into the amino conduritol derivative **173** as mentioned in the corresponding section.

In this way the formation of oxazine **171** served a multiple purpose. It provided a derivative with two chemically different bromine atoms that allowed for a selective coupling with borate **236**. In addition, because of the high stereoselectivity of the Diels-Alder cycloaddition the reaction set the four stereocenters of narciclasine (C2, C3, C4, and C4a) in the correct configuration (Figure 54). Finally, oxazine **171** was used in the chemical verification of the absolute stereochemistry of metabolite **167** (Figure 51).

Further study of this reaction allowed us to optimize a one-pot procedure for the protection of diol **167** and contiguous cycloaddition. Dimethoxypropane (DMP) is used in large excess for acetonide formation and often can be considered as a cosolvent in the reaction. In this case, we performed the reaction in neat DMP and this solvent was also suitable for the Diels-Alder cycloaddition. After verifying complete reaction of diol **167** we added the periodate and the carbamic acid (dissolved in methanol) at 0°C and allowed the reaction to warm up. The advantages of this procedure are that it shortened the preparation and avoided the isolation of acetonide **169** that is prompt to quick dimerization in the pure state.[44,148]

Our initial plan was to follow the previous work of Keck *et al.*[45,149] and Hudlicky *et al.*[35,42] and open the oxazine bridge with aluminum amalgam in order to obtain a brominated amino conduritol derivative such as **174**. Exploring this route, we found the same type of overeduction problems reported by Hudlicky and Olivo in the synthesis of lycoricidine.[35] Overeduction of the vinylic bromine on C10a (narciclasine numbering) took place under such conditions and the regular (debrominated) conduramine derivative **173** was the product isolated in a 99:1 ratio (HPLC). We studied the possibility of reducing the oxazine to unsaturated ketone **237**. Tributyltin hydride or *tris-*

trimethylsilylsilane (TTMSS) are suited for this transformation but cannot be applied to oxazine **171** since dehalogenation is unavoidable under such conditions. Conversely, $Mo(CO)_6$ [150-153] cleanly reduced dibrominated oxazine **171** to the corresponding bromo ketone **237** with concomitant (and interesting) cleavage of the acetonide protecting group (Figure 65). Because the mechanism of the cleavage with $Mo(CO)_6$ does not involve radicals formation but is rather a metal insertion process[150] it can be run safely in the presence of vinylic halides.



Conditions: (a) Al(Hg), THF-H_2O, 0°C to r.t., 4 h. (b) $MoCO_6$, MeCN-H_2O, reflux, 2h. (c) $Zn(BH)_4$, DME-THF, -10°C, 1 h.

Figure 66. Cleaving of the NO bridge in oxazine **171**.

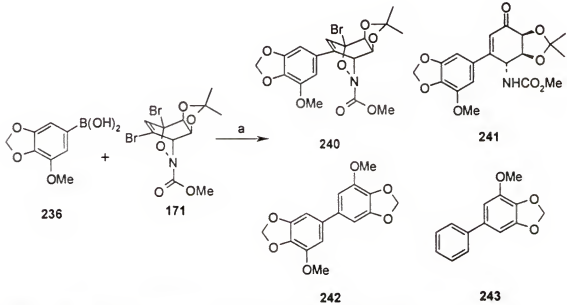
Since the $Mo(CO)_6$ reaction yielded an α-hydroxy ketone (**237**) we explored the possibility of directed hydride reduction by means of $Zn(BH_4)_2$ [154,155] or $NaB(AcO)_3H$.[156] Zinc borohydride is a non-commercial reagent that can be easily prepared from sodium

borohydride and anhydrous zinc chloride. It has an interesting selectivity that makes it a suitable choice in many cases. For example, it can readily reduce free carboxylic acids to the corresponding alcohols, a reaction that is often laborious. Because of the strong chelating properties of the zinc cation, the reagent has been applied to attain *anti* selectivity in the reduction of acyclic α-hydroxyketones.[157]

Sodium triacetoxyborohydride has been exploited for the reduction of β-hydroxy ketones with high selectivity, and it is plausible that the reagent could effectively deliver a hydride to a α,β-dihydroxy ketone like **237**. In our experiments none of these reagents achieved high selectivity. To date this reaction has provided triol **238** in only a disappointing 10% diasteromeric excess as measured by HPLC (Figure 66).

To circumvent the problem of overreduction of the bromine atom on C10a, we decided to couple the aromatic portion of the alkaloid (borate **236**) directly to oxazine **171** and postpone the bridge opening to a later stage in the synthesis. The coupling was performed under the standard Suzuki-Miyaura conditions[112] worked out during the previous projects. In this case the reaction proceed only in fair yield (30%) and oxazine **240** was isolated together with 10-15 % of ketone **241** and 20-25 % of biphenyl compounds identified as **242** and **243** (Figure 67). Ketone **241** could be formed through a palladium insertion-type mechanism similar to regular cleavage of the NO bond by $Mo(CO)_6$ discussed above. The presence of biphenyls of the type of **242** and **243** is often discussed in the Suzuki coupling. It was first observed by Miyaura and Suzuki[113] and has been studied in detail by Marcuccio.[114] These types of byproducts are produced by homocoupling of the borate ligands or by transfer of a phenyl group from the catalyst to the borate. Generally they are associated with the coupling of low reacting halide

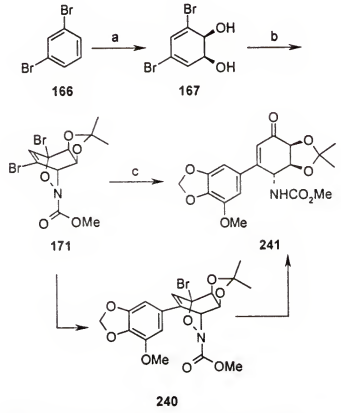
partners in the coupling reaction. Oxazine **171** was not a highly reactive partner, but behaved reasonably well considering the high degree of functionalization present in the molecule. To the best of our knowledge, this is the first example of Suzuki coupling of a halo-oxazine and a phenyl borate.



Conditions: (a) 0.03% eq. $Pd(PPh_3)_4$, aq. Na_2CO_3, PhH-EtOH, reflux, 3h. 30%.
Figure 67. Suzuki coupling.

Surprisingly, oxazine **240** was resistant to aluminum amalgam reduction under Keck conditions, while stronger reducing agents (sodium amalgam or H_2/Pd) led to fully saturated products. This result closed the direct reduction pathway to the α-hydroxy compound; therefore, we chose to transform **240** into unsaturated ketone **241** with *tris*-trimethylsilyl silane (TTMSS). Since we have observed the formation of **241** during the Suzuki coupling we recognized here another opportunity to optimize a one pot procedure. Guided by that observation we decided to add acetonitrile and $Mo(CO)_6$ directly to the

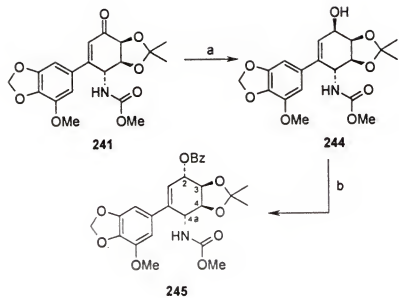
Suzuki reaction mixture after the coupling was finished. Heating of this mixture for 10 hours afforded ketone **241** in 45% yield. In this fashion we were able to optimize a preparation of the advanced intermediate **241** in only three operations from *m*-dibromobenzene (Figure 68).



Conditions: (a) DMP, TsOH, r.t. 10 min., then $MeCO_2NHOH$, Bu_4NIO_4, 0ºC to r.t., 12 h. (c) 0.03% eq. $Pd(PPh_3)_4$, aq. Na_2CO_3, PhH-EtOH, reflux, 3h then $MoCO_6$, reflux, 12h. 40%.

Figure 68. Three operations synthesis of ketone **241** from a non-chiral starting material.

In order to set the stereochemistry at C2 (narciclasine numbering), we applied the known Luche reduction followed by Mitsunobu inversion sequence as reported by Chida in his lycoricidine preparation.[36,37] This procedure gave cleanly the desired α-benzoate **245** in 65% yield from ketone **241** (Figure 69).



Conditions: (a) $NaBH_4$, $CeCl_3$, MeOH, 0 °C, 80%; (b) BzOH, Bu_3P, DEAD, THF, rt, 65%.

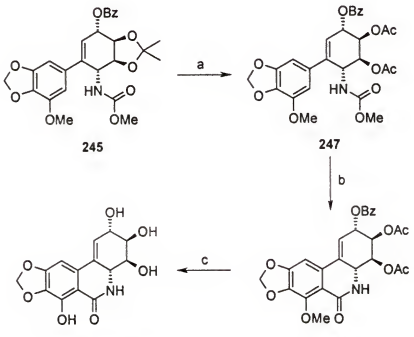
Figure 69. Setting of the stereocenters in narciclasine.

A modification of the Bischler-Napieralski reaction reported by Banwell *et al.*[158] and applied with success by the same author in simplified models of phenanthridone alkaloids,[159] was chosen to close ring B of the target. This interesting variation of the traditional conditions uses a 5:3 mixture of triflic anhydride and DMAP instead of the harsh $POCl_3$ to attain cyclization. It has been applied successfully to sensitive molecules not only by Banwell *et al.*[159] but also by Hudlicky *et al.* in his 7-deoxypancratistatin preparation.[22,27] Although the conditions are mild, they are not compatible with acid sensitive protecting groups such as acetonides. Therefore, in order to apply the Banwell conditions some protective group manipulation was required (Figure 70). The acetonide **245** was removed by action of an acidic resin in methanol. This method is convenient because the diols are generally very soluble in methanol and simple filtration of the resin

renders a essentially pure solution of the product. At this stage we did not purify diol **246**. Instead, we evaporated the solvent and treated the crude with acetic anhydride and pyridine. The resulting diacetate **247** was obtained in 90% yield over the two steps (performed as a single operation). Compound **247** was submitted to the conditions of Banwell and afforded phenanthridone **248** in 40% yield. The rest of the material was transformed into phenolic compounds due to elimination driven, perhaps, by the excess of triflic anhydride. The particular ratio of Tf_2O/DMAP : 5/3 has been empirically determined by Banwell *et al.*[158] We tried an equimolecular mixture and no cyclization was observed. The application of the reaction to other substrates (including acetonides **244** and **245**, and ketone **241**) afforded only phenolic material. Although the closure could render two isomers (phenanthridones **248** and **249**) we never observed the formation of two products. Apparently the 7-methoxy group is directing the reaction and activating the **C6a** position. Distinction between the two isomer **248** and **249** by spectroscopic methods was almost impossible and was out of the question with only minute amounts of one isomer in hand. At this stage of the synthesis it was wiser to deprotect and compare the final product with the reported data for narciclasine (**2**) and its known derivative 7-*O*-methylnarciclasine (**250**).

Removal of the ester groups in **248** was done by treating with a basic Amberlyst resin in dilute methanol solution. Dilution was essential because the solubility of the triol **249** was unknown. This reaction worked efficiently and a polar fluorescent solid was formed. This compound had an identical ^{1}H NMR spectrum and a matching optical rotation ($[\alpha]_D^{26}$ = 204 (c = 0.3, DMSO)) with the corresponding for the same compound

prepared by methylation of natural narciclasine using Piozzi's procedure[28] (diazomethane in ethanol, 5 five days, 50%) ($[\alpha]_D^{26}$ = +219 (c = 1.0 in DMSO).



Conditions: (a) Dowex 50X8-100, MeOH, rt; then Ac_2O, py, DMAP, rt, 70%; (b) Tf_2O, DMAP, CH_2Cl_2, 0 °C, 40%; (c) Amberlyst A21, MeOH, rt; then LiCl, DMF, 120 °C, 10-20%.

Figure 70. Final closure and deprotection.

Cleavage of the methyl ether on the C7-OH proved to be cumbersome. After several trials with the reported conditions of Trost and Pulley (LiI, DMF, 80°C, several hours) for deprotection of 7-*O*-methylpancratistatin,[48] we only observed degradation products. After a personal communication with Dr. Pulley we obtained the correct procedure that used LiCl in DMF at 120°C for two hours. This procedure performed on (1.0 ± 0.1) mg of **249** afforded a different polar compound that presented a strong yellow green fluorescence. Purification afforded (0.3 ± 0.1) mg of a compound that showed an

identical ^{1}H NMR spectrum and a matching optical rotation with the literature data for narciclasine ($[\alpha]_D^{23}$ 130 (c = 0.03 in DMSO), lit.[21] 141.8) and presented a TOCSY spectrum fully consistent with structure **2**.

Overall, we have completed a total synthesis of narciclasine in twelve steps carried out as only eight individual chemical operations from *m*-dibromobenzene (fourteen from *o*-vanillin) (Figure 71). This was the second synthesis of narciclasine to be published and it was eleven steps shorter than the previous preparation.

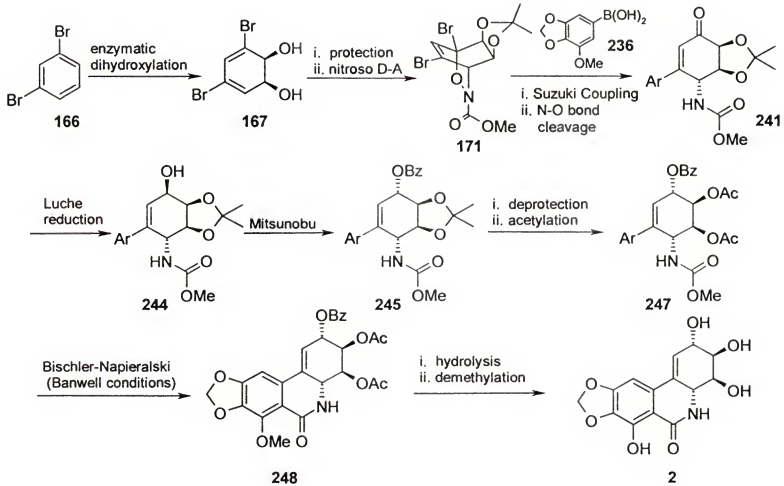


Figure 70. Summary of narciclasine synthesis.

CHAPTER 4
CONCLUSION

In the course of this research we have isolated and identified four new metabolites of toluene dioxygenase-mediated oxidation of aromatics. The four metabolites isolated have been fully characterized and their absolute stereochemistry confirmed by either spectroscopic methods or synthesis. The main purpose of the biotransformation work was not the isolation *per se* but the search for new optically pure synthons to be used in organic preparations. There is a basic philosophical distinction between this work and many previous reports in the field. Previously, chemists would look at those metabolites already isolated and identified, and envision them as suitable starting materials for synthesis. The approach of the synthetic chemist was essentially based in the recognition of a known substrate as a key piece in the skeleton of the target. In a complementary approach, the chemists and biologists working on biotransformations prepared new metabolites and added them to the chiral pool. It was not common that the metabolite would be conceived on paper before it was actually isolated. In our approach we have inverted the order of events by *designing the approach to the target first*, disconnected to an ideal starting material, and then tested the biotransformation. In that way biotransformation becomes a "custom tool" that can prepare the required synthon following the synthetic demands. There are very few examples of this type of thinking

and they all came from the Hudlicky group. Remarkable examples of these ideas are the approaches to morphine from diols **252**[137] and specially **254**[50,51] (Figure 72).

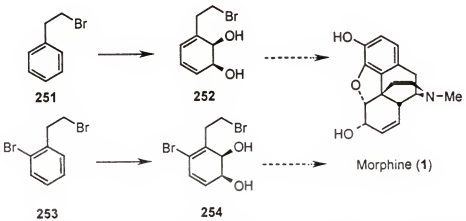


Figure 72. Two previous examples of custom biotransformations projected for use in synthesis.

We have used diols **143** and **144** in the preparation of unnatural amino acids of interesting structure. This work has also been useful to expand and test the scope of a new variation of the Claisen rearrangement discovered by Kazmaier.[60,61] We applied the Chelated-enolate Claisen rearrangement to substrates of greater complexity and contributed to study the scope and limitations of this new reaction. The rearranged products and specifically the product ultimately derived from a 2,3-dimethoxy aromatic substrates are potentially applicable in a synthesis of morphine that has not yet been reduced to practice. The poor metabolization of the dimethoxy substituted arenes as well as the decreased selectivity observed in the rearrangement have momentarily delayed the approach. Some of the problems observed could be overcome by redesigning the synthesis. One possibility is the preparation of ketone **234** as presented in Figure 61 (see

previous chapter). Another options imply a more radical redesign. A perhaps-plausible example of a new route is sketched out in Figure 73.

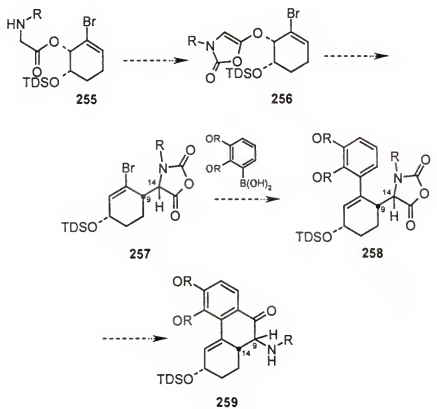


Figure 73. Alternative approach to morphine alkaloids.

Amino ester **255** could be prepared by standard methods as it was described for analogous compounds. Then it could be transformed into oxazolone **255** by reaction with diphosgene. This compound has some resemblance with the classical Steglich oxazole rearrangement[55,56] discussed in Chapter 2. Therefore, it is conceivable that it could rearrange to afford **257** in parallel with Steglich results. This rearrangement does not necessitate strong bases and consequently is more compatible with the presence of vinylic halogens in the molecule, an issue that had plagued our yields (see Table 9, entry 2). The

rearranged product could be coupled at a later stage of the synthesis and thus the preparation would be less borate-intensive. This is relevant because of the laborious preparation of the required bromide **148**. Finally, the coupled compound **258** could be studied for Friedel-Crafts type reactions directly or after a suitable modification.

The preparation of narciclasine worked as a corollary to this dissertation since it proved the feasibility of the "biphenyl type approach" (Figure 2) to the amaryllidaceae alkaloids. A rapid increase in complexity was achieved in the sequence by the combination of Suzuki coupling and biotransformations demonstrating the complementary nature of enzymatic methods and organometallic chemistry. The brevity of the preparation compensated for the low yield obtained in several steps but still more work is required to achieve a practical preparation. An important merit of this synthesis is that opened the doors for a related strategy towards the enantiomers of narciclasine and lycoricidine. A brief description of such strategy is outlined in Figure 74.

Coupling of a borate of type **236** with the known metabolite **168** could afford the required "biphenyl diol". Alternatively this coupling could be effected latter in the synthesis. Manipulation of the diol according to well established procedures should render epoxide **259** that could be opened and acetylated to obtain diol **260** after removal of the acetonide group. Inversion of the free allylic diol in **260** perhaps without protection of the homoallylic hydroxyl group could provide azide **261** that can be elaborated further towards the enantiomers **264** and **264** via a carbamate of type **262**.

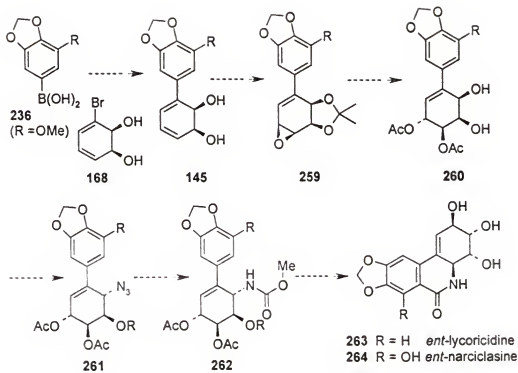


Figure 74. A possible approach to the enantiomers of lycoricidine and narciclasine.

In summary, the combination of biotransformations and traditional synthetic organic chemistry resulted in a synergic effect that allowed for the design of preparations with brevity and efficiency unattainable for any of the techniques considered individually. It is anticipated that the continued research in the field will improve and expand these modest achievements.

CHAPTER 5
EXPERIMENTAL DETAILS

5.1. General Procedures

All non-hydrolytic reactions were carried out in a argon atmosphere with standard techniques for the exclusion of air and moisture. Glassware used for moisture sensitive reactions was flame dried under reduced pressure or dried overnight at 80°C. Tetrahydrofuran, benzene, and diethyl ether were distilled from sodium benzophenone ketyl. Dichloromethane was distilled from calcium hydride. Flash column chromatography was performed on Merck silica gel (grade 60, 230-400 mesh). Microbial oxidations were carried out in a B. Braun Biostat E fermentor with a 12 L vessel. ^{1}H NMR spectra were recorded at 300 (Varian Gemini 300 or Varian VXR 300) or 500 MHz (Varian Unity 500), 13C NMR were recorded at 75 or 126 MHz. Infrared spectra were recorded in a Perkin Elmer 1600 FTIR spectrophotometer. Optical rotations were recorded in a Perkin Elmer 341 polarimeter. HPLC analyses were performed in a Hitachi L-6000 chromatograph using various acetonitrile/water mixtures as eluents. Melting points were recorded on a Thomas Hoover unimelt apparatus and are uncorrected. Atlantic Microlabs, Norcross, GA, performed all elemental analyses. Whenever possible elemental analysis was chosen in place of HRMS. HMRS was taken when the analysis failed more than once or sufficient material was not available.

5.2. New Metabolites

The biooxidation was performed following a standardized procedure. An excellent description of the method, aimed to the organic chemists, has been recently described by Hudlicky *et al.*[160] A brief summary of that procedure is presented below.

E. coli JM109 (pDTG601A) was grown overnight at 35ºC in an enriched medium containing ampicilin. The preculture was then transferred to a 12 L fermentor containing 8L of a similar medium and the cells are grown to an OD = 70 (λ=660 nm). The substrates were added to the culture and their metabolic transformation was monitored observing the oxygen consumption and carbon dioxide production by the culture. Diol production was checked by measuring a characteristic absorbance peak in the UV region (λ=220 nm). After all metabolic activity ceases (or no more diol formation is observed) the fermentation was stopped and the pH was adjusted to 7.5 with ammonium hydroxide. The cells were separated from the broth by centrifugation and the resulting clear solution was saturated with sodium chloride and extracted with base-washed ethyl acetate. The organic layer was dried with anhydrous magnesium sulfate and the solvent evaporated. The crude diols were purified by recrystallization (dichloromethane/pentane) to yield the pure compounds as yellowish solids.

Note: some of these metabolites are unstable at room temperature. In such cases the usual procedure is to determine only minimal data (^{1}H NMR and optical rotation) and transform the metabolite into a stable derivative that is fully analyzed.

(1*S*, 2*S*)-3-Bromo-4,5-difluoro-3,5-cyclohexadiene-1,2-diol (127). R_f = 0.4 (hexanes/ethyl acetate 1:1) mp 104.5-105.5 ºC (ethyl ether) $[\alpha]_D^{28}$ +34.6 (*c* 0.49, MeOH).

IR (KBr) 3910 (w), 1630 (m), 1380 (m), 1220 (s), 1090 (s), 1015 (s). ^{1}H NMR ($CDCl_3$) δ 5.12 (m, 1H), 4.60 (br s, 1H), 4.47 (br s, 1H), 2.66 (br s, 1H), 2.40 (br s, 1H). ^{13}C NMR (C_3D_6O) δ 148.5 (dd, *J* = 259.0, 26.3 Hz), 148.4 (dd, *J* = 258.6, 28.2 Hz), 109.2 (dd, *J* = 9.9, 1.5 Hz), 106.8 (dd, *J* = 13.3, 3.4 Hz), 73.0 (d, *J* = 2.3 Hz), 67.7 (d, *J* = 8.4 Hz), ^{19}F-NMR ($CDCl_3$): δ (ppm) -124.25 (broad s), -132.84 (broad s.). MS *m/e* (relative intensity) 228(24, $M^+(C_6H_5O_2{}^{81}BrF_2)$), 226(19, $M^+(C_6H_5O_2BrF_2)$); 130(16); 127(21); 101(100). HRMS Calcd. for $C_6H_5O_2BrF_2$ 225.9441 Found 225.9477. Anal. Calc. for $C_6H_5BrF_2O_2$: C, 31.75; H, 2.22; Found: C, 31.46; H, 2.19.

3-(2-Methoxyphenyl)-(1*S*, 2*R*)-3,5-cyclohexadiene-1,2-diol (143). Yield: 2.5 g/L. White crystalline solid (unstable); mp 77-78°C; R_f = 0.3 hexanes/ethyl acetate, 50:50; $[\alpha]_D^{30}$ +160° (c 1.74, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25 (m,, 2H), 6.95 (m, 2H), 6.07 (m, 2H), 5.92 (m, 1H), 4.51 (bs, 1H), 4.42 (bd, *J* = 5 Hz, 1H), 3.86 (s, 3H), 3.31 (bs, 1H), 2.81 (bs, 1H). ^{13}C NMR δ (75 MHz, $CDCl_3$) 156.3, 139.2, 130.8, 129.9, 129.1, 124.3, 123.9, 121.3, 110.8, 69.7, 69.4, 55.7.

3-(2,3-Dimethoxyphenyl)-(1*S*, 2*R*)-3,5-cyclohexadiene-1,2-diol (144). Yield: 0.8 g/L. Yellow oil (unstable), R*f* =0.3 hexane/ethyl acetate, 50:50; $[\alpha]_D^{24}$ +62.5° (c 0.8, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.07 (t, *J* = 8.0 Hz, 1H), 6.89 (td, *J* = 7.0, 1.4 Hz, 2H), 6.12 (m, 2H), 6.02 (m, 1H), 4.49 (bs, 2H), 3.89 (s, 3H), 3.84 (s, 3H), 3.38 (bs, 1H), 2.58 (bs, 1H).

3,5-Dibromo-(1*S*, 2*S*)-3,5-cyclohexadiene-1,2-diol (167). Yield: 3-4 g/L. White needles (Dichloromethane) (unstable); Rf = 0.4 (hexanes/ethyl acetate : 50/50); mp: 80-81 °C. $[\alpha]_D^{25}$ = +21.3 (C = 1.1, acetone); IR (KBr): 3255, 1279, 1113, 1027; ^{1}H NMR

($CDCl_3$, 300 MHz) δ 6.43 (dd, J = 1.5, 0.9 Hz. 1H), 6.25 (dd, J = 4.2, 1.5 Hz, 1H), 4.41 (dd, J = 6.3, 4.2 Hz, 1H), 4.29 (dd, J = 6.3, 0.9 Hz, 1H), 2.80 (bs, 2H); ^{13}C NMR (d^6-acetone, 300 MHz) δ 131.7, 130.3, 129.9, 114.9, 72.1, 71.0; MS (-)ESI CH_3COO^-: 271(^{81}Br+^{81}Br (M-H)$^-$) 269(^{81}Br+^{80}Br (M-H)$^-$), 267(^{80}Br+^{80}Br (M-H)$^-$); Anal. Calcd. for: $C_6H_6Br_2O_2$ –H_2O (phenol) C 28.61, H 1.60. Found: C 28.22, H 1.89.

5.3. Unnatural Amino Acids

5.3.1. General Procedure for Diimide Reduction of the Diene Diols.

Potassium azodicarboxilate (PAD) (2.0 eq.) was added in small portions with vigorous stirring to an ice cold solution of the diene diol (1.0 eq.) in methanol (10 ml/mmol), followed by dropwise addition of a solution of acetic acid (7.0 eq.) in methanol. The solution was allowed to slowly warm to room temperature. Saturated $NaHCO_3$ solution was added until the solution become slightly basic (pH=8). The methanol was removed under reduced pressure and the remaining aqueous solution was extracted with ethyl acetate (4 X 30 mL). The combined organic layers were dried (magnesium sulfate) and the solution was filtered through a bed of silica gel. The solvent was removed under reduced pressure to yield a white solid. (85-90%) The compounds were further purified by recrystallization (white needles from ethyl ether/hexanes) for analytical purposes.

Note: The toluene derivative diol **184** is known compound[161]

3-Phenyl-(1*S*,2*R*)-3-cyclohexene-1,2-diol. (186). White crystalline solid; mp 98-99 °C; R_f = 0.3 hexanes/ethyl acetate, 50:50; $[\alpha]_D^{23}$ -101.4° (c 0.8, $CHCl_3$); IR (KBr) 3260, 3025, 2924, 1499, 1127, 1081, 757; ^1H NMR (300 MHz, C_2D_6O) δ 7.56 (m, 2H),

7.25 (m, 3H), 6.16 (dd, J = 3.2, 4.6 Hz, 1H), 4.49 (t, J = 4.3 Hz, 1H), 3.90 (d, J = 5.7 Hz, 1H), 3.85 (d, J = 5.1 Hz, 1H), 3.74 (ddd, J = 3.4, 6.8, 15 Hz, 1H), 2.40-2.14 (m, 2H), 1.87 (ddd, J = 6.4, 12.0, 22.7 Hz, 1H), 1.74-1.64 (m, 1H); ^{13}C NMR (75 MHz, C_2D_6O) δ 141.38, 138.33, 128.54, 127.81, 127.06, 126.29, 70.13, 68.15, 25.53, 24.94; LRMS (FAB) *m/z* (rel intensity) 190 (M^+, 25), 173 (100), 154 (25), 136 (20), 91 (25); HRMS calcd. for $C_{12}H_{14}O_2$: 190.0994. Found: 190.0993; Anal. calcd. for $C_{12}H_{14}O_2$: C, 75.76; H, 7.72. Found: C, 75.80: H, 7.42.

3-(2-Methoxyphenyl)-(1*S*,2*R*)-3-cyclohexene-1,2-diol (154). White crystalline solid; mp 82.5-83.0°C; R_f= 0.3 hexanes/ethyl acetate, 50:50; $[\alpha]_D^{23}$ -80.7° (c 1.0, $CHCl_3$); IR ($CHCl_3$) 3564, 3020, 2937, 2840, 1598, 1489 1464, 1436, 1246, 1222, 1206; ^{1}H NMR (300 MHz, CDCl3) δ 7.19 (m, 1H), 7.09 (dd, J =7.4, 1.4Hz, 1H), 6.85 (m, 2H), 5.79(t, J = 3.7 Hz, 1H), 4.42 (bd, J = 2.8 Hz, 1H), 3.88-3.79 (m, 1H), 3.76 (s, 3H), 2.61 (bs, 2H), 2.24 (m, 2H), 1.78 (m, 2H); ^{13}C NMR (75 MHz,$CDCl_3$) δ 169.5, 156.20, 137.35, 130.98, 130.39, 128.67, 121.12, 110.60, 69.29, 68.65, 55.53, 25.05, 24.40. (LRMS (CI/CH_4) *m/z* (rel intensity) 220 (M^+, 12), 203 (100), 175 (8); HRMS calcd. for $C_{13}H_{16}O_3$: 220.1099. Found: 220.1098.

3-(2,3-Dimethoxyphenyl)-(1*S*,2*R*)-3-cyclohexene-1,2-diol (155). White crystalline solid; mp 66.0–67.0°C; Rf =0.3 hexane/ethyl acetate, 50:50; $[\alpha]_D^{23}$-62.9 (c 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.03 (t, J = 17.7 Hz, 1H), 6.87 (d, J =7.1 Hz, 1H), 6.77 (dd,J =7.4, 0.8 Hz, 1H), 5.89 (t, J =3.6 Hz, 1H), 4.42 (bs, 1H), 3.94–3.89 (m, 1H), 3.87 (s, 3H), 3.70 (s, 3H), 2.60 (bs, 2H), 2.30 (m, 2H), 1.88 (m, 2H); 13 C NMR (75 MHz, CDCl3) δ 152.5, 145.8, 136.6, 135.8, 130.5, 124.5, 122.5, 111.6, 69.3, 69.0, 61.0,

55.8, 25.2, 24.2; LRMS (CI/CH4) *m/z* (rel intensity) 250 (M^+, 100), 232 (35), 206 (93); HRMS calcd for $C_{14}H_{18}O_4$: 250.1205. Found: 250.1205.

5.3.2. General Procedure for Suzuki Coupling.

To a round bottom flask under an argon atmosphere was added $Pd(PPh_3)_4$ (1.32g, 0.001 mol). This was followed by addition of 50 mL dry benzene. A solution of the bromide (7.40 g, 0.040mol) dissolved in 10mL of ethanol was then added to the reaction flask. This was followed by the addition of Na_2CO_3 (36 mL, 2M) to the mixture. Dimethoxyphenyl boronic acid (8.40g, 0.046 mol) was dissolved in 50 ml of dry benzene and was then added to the reaction mixture which was allowed to reflux for 6h. The reaction was quenched with water and the product extracted with ethyl acetate (3 X 50 mL). The organic layers were combined, washed with brine and dried over anhydrous $MgSO_4$. After filtration, the solvent was removed, the crude product introduced onto a silica gel column, and eluted with ethyl acetate: hexane (1/3) to obtain 7.10 g (83%) of **155** as white crystals (**154**, 75%)

154: $[\alpha]_D^{25}$ -76.6 (c 1.0, $CHCl_3$)

155: $[\alpha]_D^{23}$ -58.3 (c 1.1, $CHCl_3$)

5.3.3. General Procedure for TDS Protection.

A solution of the diol (1.0 eq.) and imidazole (1.3 eq) in DMF (0.9 ml/mmol) was prepared in a dry round bottom flask under an argon atmosphere. The flask was cooled to -12°C and TDSCl was added with very slow stirring. The flask was stored at -18°C for 12 hours. The solution was diluted with ethyl ether and brine was added. After separation the aqueous solution was extracted with ethyl ether (2 X 30 mL) and the combined organic layers were washed with brine (1 X 5 mL), $CuSO_4$(aq) 10% (3 X 3 mL), to remove the

imidazole and one more time with brine (5 mL). The extract was dried (magnesium sulfate) and the solvent evaporated. Purification by column chromatography (silica gel, hexanes:ethyl acetate, 99:1) afforded the protected compounds (80-90%).

Note: The toluene derivative alcohol **187** is a known compound[161]

6-Phenyl-2-dimethylthexylsilyloxy-(1*R*, 2*S*)-5-cyclohexen-1-ol (189). Yellow oil; R_f = 0.6 hexanes:ethyl acetate, 80:20; $[\alpha]_D^{26}$ +220° (c 0.4, $CHCl_3$); IR ($CHCl_3$) 2961, 2872, 1657, 1466, 1378, 1255, 1090, 935, 877; ^1H NMR (300 MHz, $CDCl_3$) δ 7.56 (m, 2H), 7.34 (m, 2H), 7.25 (m, 1H), 6.22 (dd, *J* = 2.9, 4.9 Hz, 1H), 4.43 (d, *J* = 2.9 Hz, 1H), 3.90 (dt, *J* = 3.8, 11.5 Hz, 1H), 2.40 (m, 1H), 2.31-2.19 (m, 1H), 2.03-1.89 (m, 1H), 1.76-1.62 (m, 2H), 0.92 (dd, *J* = 6.8, 2.2, Hz, 6H), 0.88 (s, 6H), 0.18 (s, 3H), 0.16 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 140.34, 136.87, 128.32, 128.00, 127.93, 126.95, 125.79, 71.17, 68.36, 34.90, 34.23, 25.14, 24.68, 20.38, 20.02, 18.63, 18.53, -2.39, -2.85; LRMS (FAB) 333 (M^+, 5), 315 (12), 231 (100), 155 (58); HRMS calcd for $C_{20}H_{33}O_2Si$: 333.2250. Found: 333.2281. Anal. calcd for $C_{20}H_{33}O_2Si$: C, 72.23; H, 9.70. Found: C, 72.34; H, 9.65.

6-Chloro-2-dimethylthexylsilyloxy-(1*S*,2*S*)-5-cyclohexen-1-ol (188) Yellow oil; R_f = 0.6 hexanes:ethyl acetate, 70:30; $[\alpha]_D^{26}$ -45.1 (c 1.2, $CHCl_3$); IR ($CHCl_3$) 3543, 2962, 2871, 1653, 1466, 1378, 1102; ^1H NMR (300 MHz, $CDCl_3$) δ 5.96 (t, *J* = 4.0, 1H), 4.03 (bs, 1H), 3.90 (dt, *J* = 3.7, 10.3, Hz, 1H), 2.76 (d, *J* = 2.9 Hz, 1H), 2.30-2.17 (m, 1H), 2.14-2.00 (m, 1H), 1.90-1.54 (m, 1H), 1.68-1.55 (m, 2H), 0.89 (dd, *J* = 1.3, 6.8 Hz, 6H), 0.86 (s, 6H); 0.15 (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 131.21, 127.94, 71.14, 70.71, 34.22, 25.11, 24.98, 24.10, 20.17, 18.61, 18.29, -2.43, -2.95; LRMS (FAB) *m/z* (rel intensity) 291 (M^+, 66), 273 (30), 205(68), 189(100); HRMS calcd for $C_{14}H_{28}O_2ClSi$:

291.1547, Found: 291.1554 Anal. calcd for $C_{14}H_{28}O_2ClSi$: C, 57.80; H, 9.35. Found: C, 58.79; H, 9.39.

6-(2-Methoxyphenyl)-2-dimethylthexylsilyloxy-(1*R*,2*S*)-5-cyclohexen-1-ol (190). Yellow oil; R_f = 0.7 hexanes:ethyl acetate, 80:20; ^{1}H NMR (300 MHz, $CDCl_3$) 7.24 (m, 2H), 6.94 (t, *J* = 7 Hz, 1H), 6.87 (d, *J* = 8.0 Hz, 1H) 5.85 (t, *J* = 3.6 Hz, 1H), 4.54 (bs, 1H), 4.44 (dt, *J* = 9.9, 3.3 Hz, 1H), 3.82 (s, 3H), 2.54 (d, *J* = 4.1 Hz, 1H), 2.42-2.16 (m, 2H), 2.02-1.86 (m, 1H), 1.76-1.58 (m, 2H), 0.90 (dd, *J* = 6.9, 1.7, Hz, 6H), 0.86 (s, 6H), 0.17 (s, 3H), 0.15 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156.57, 137.35, 130.63, 129.40, 128.31, 120.74, 110.49, 70.61, 68.82, 55.36, 34.27, 25.45, 24.93, 24.23, 20.39, 20.23, 20.11, 18.63, 18.54, -2.48, -2.98

Note: Carried out to the next step without purification

6-(2,3-Dimethoxyphenyl)-2-dimethylthexysilyoxy-(1R,2S)-5-cyclohexen-1-ol (191). Yellow oil; Rf = 0.7 hexane: ethyl acetate, 80: 20; $[\alpha]_D^{32}$ -59.3 (c 1.0, $CHCl_3$); IR (KBr/ cm^{-1}): 3245, 2959, 1470, 1259, 1108, 1011; ^{1}H NMR ($CDCl_3$) δ: 7.0 (t, *J* = 7.2 Hz, 1H), 6.8 (d, *J* = 7.7 Hz, 2H), 5.9 (t, *J* = 3.6 Hz, 1H), 4.4 (bs, 1H), 4.0 (dt, *J* = 10.2, 3.3 Hz, 1H), 3.8 (s, 3H), 3.7 (s, 3H), 2.6 (d, *J* = 4.1 Hz, 1H), 2.4 - 2.3 (m, 1H), 2.2 - 2.1 (m, 1H), 2.0 - 1.9 (m, 1H), 1.7 - 1.6 (m, 2H), 0.9 - 0.8 (m, 14H), 0.1 (d, *J* = 5.5 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ: 152.6, 136.3, 136.0, 129.7, 123.9, 122.4, 111.4, 70.8, 69.2, 60.6, 55.8, 34.2, 25.4, 24.9, 24.3, 20.4, 20.2, 20.1, 18.6, 18.5, - 2.5, - 2.9; HRMS: $C_{22}H_{36}O_4Si$ (M+1) Calcd. 393.2383, Found: 393.2479

5.3.4. General procedure for DCC coupling.

A solution of BOC-glycine (1.2 eq.), DCC (1.5 eq.), DMAP (0.1 eq.) in dichloromethane (10 ml/mmol) was cooled to 0ºC and a solution of the TDS protected diol (1.0 eq.) in dichloromethane (2 ml) was added. The cloudy reaction mixture was stirred overnight while it was allowed to reach room temperature. The solution was diluted with ethyl ether and filtered through a bed of silica gel to remove the precipitate of dicyclohexylurea. Removal of the solvent and chromatography (silica gel, hexanes:ethyl acetate, 90:10) of the residue, afforded the pure amino esters as thick colorless oils (70-75%).

6-Phenyl-2-dimethylthexysilyloxy-(1*R*,2*S*)-5-cyclohexen-1-yl-*N*-*tert*-(butyloxycarbonyl)glycinate (194). Colorless oil; R_f= 0.7 hexanes:ethyl acetate, 80:20; $[\alpha]_D^{24}$ -89.4° (c 1.0, $CHCl_3$); IR ($CDCl_3$) 3446, 2961, 2870, 1743, 1712, 1508, 1380, 1354, 1165, 1110; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37 (bd, *J* = 7.3 Hz, 2H), 7.30 (m, 2H), 7.24 (m, 1H), 6.30 (bs, 1H), 6.07 (bs, 1H), 4.95 (bs, 1H), 3.96 (dt, *J* = 3.7, 11.7 Hz, 1H), 3.86 (ddd, *J* = 4.9, 18.6, 38.0 Hz, 2H), 2.44 (m, 1H), 2.35-2.25 (m, 1H), 1.89 (ddd, *J* = 5.9, 11.7, 23.9 Hz, 1H), 1.75-1.69 (m, 1H), 1.63-1.58 (m, 1H), 1.42 (s, 9H), 0.87 (dd, *J* = 1.0, 6.8 Hz, 6H), 0.83 (s, 3H), 0.82 (s, 3H), 0.14 (s, 3H), 0.13 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 134.21, 130.59, 128.49, 127.37, 125.58, 70.25, 69.85, 42.57, 34.10, 28.30, 25.60, 25.30, 24.84, 20.17, 20.09, 18.51, 18.48, -2.87, -2.93; LRMS (FAB) *m/z* (rel intensity) 490 (M^+, 2), 448 (3), 422 (2), 315 (40), 176 (100); HRMS calcd for $C_{27}H_{44}O_5NSi$: 490.2989. Found: 490.2975. Anal. calcd for $C_{27}H_{44}O_5NSi$: C, 66.22; H, 8.85; N, 2.86. Found: C, 66.92; H, 9.13; N, 2.68.

6-Methyl-2-dimethylthexysilyloxy-(1*R*,2*S*)-5-cyclohexen-1-yl-*N*-*tert*-(butyloxycarbonyl)glycinate (192). Colorless oil; R_f = 0.7 hexanes:ethyl acetate; $[\alpha]_D^{26}$ –76.1° (c 0.8, $CHCl_3$); IR ($CDCl_3$) 3453, 3001, 1750, 1690; ^1H NMR (300 MHz, $CDCl_3$) δ 5.61 (bs, 1H), 5.33 (bd, *J* =3.8 Hz, 1H), 5.01 (bs, 1H), 3.87 (m, 3H), 2.25-1.94 (m, 2H), 1.79-1.5 (m, 3H), 1.42 (s, 9H), 0.83 (s, 3H), 0.82 (s, 3H), 0.78 (d, *J* = 1.8 Hz, 6H), 0.08 (s, 3H), 0.06 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170,12, 155.33, 130.22, 127.44, 79.53, 72.84, 69.13, 42.46, 40.00, 28.16, 26.01, 24.66, 24.28, 22.48, 20.42, 20.03, 19.96, 18.35, 18.13, 13.94, -3.02, -3.15; HRMS calcd for $C_{22}H_{42}O_5NSi$: 428.2832. Found: 428.2802.

6-Chloro-2-dimethylthexysilyloxy-(1*R*,2*S*)-5-cyclohexen-1-yl-*N*-*tert*-(butyloxycarbonyl)glycinate (193). Colorless oil; R_f = 0.7 hexanes:ethyl acetate, 80:20; $[\alpha]_D^{24}$ -93° (c 0.7, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$), 6.06 (dd, *J* = 4.9, 2.9 Hz,1H), 5.54 (d, *J* = 3.9 Hz, 1H), 5.09 (bs, 1H), 3.96 (m, 3H), 2.35-2.07 (m, 2H), 1.85-1.62 (m, 2H), 1.62-1.53 (m, 1H), 1.45 (s, 9H), 0.87 (s, 3H), 0.85 (s, 3H), 0.80 (d, *J* = 1.7 Hz, 6H), 0.10 (d, *J* = 4.6 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ), 169.57, 155.24, 130.38, 127.38, 79.54, 72.64, 69.10, 42.30, 33.98, 28.17, 25.59, 24.71, 24.28, 20.00, 19,95, 18.35, -3.09, -3.16; HRMS calcd for $C_{21}H_{39}O_5NSiCl$: 448.2286. Found: 448.2293.

6-(2-Methoxyphenyl)-2-dimethylthexysilyloxy-(1*R*,2*S*)-5-cyclohexen-1-yl-*N*-*tert*-(butyloxycarbonyl)glycinate (195). Colorless oil; R_f = 0.5 hexanes:ethyl acetate, 80:20; $[\alpha]_D^{26}$ +68.2 (c 0.9, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 7.21 (m, 1H), 7.15 (bd, *J* = 8Hz, 1H), 6.85 (m, 2H), 6.12 (bs, 1H), 5.90 (bs, 1H), 4.84 (bs, 1H), 4.16 (m, 1H), 3.80 (s, 3H), 3.78-3.64 (m, 2H), 2.43-2.18 (m, 2H), 1.89 (ddd, *J* = 5.9, 11.7, 23.9 Hz, 1H), 1.75-1.69 (m, 1H), 1.63-1.58 (m, 1H), 1.42 (s, 9H), 0.87 (dd, *J* = 1.0, 6.8 Hz,

6H), 0.83 (s, 3H), 0.82 (s, 3H), 0.14 (s, 3H), 0.13 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ169.62, 156.55, 155.22, 134.61, 131.16, 130,40, 129.19, 128.61, 120.59, 110.33, 79.41, 71.73, 68.56, 55.28, 42.34, 34.19, 28.21, 26.01, 24.83, 24.37, 20.21, 20.12, 18.48, 18.42. -3.10. HRMS calcd for $C_{28}H_{46}O_6NSi$ (M^+ - TDS group): 520.3094. Found: 520.3176.

6-(2,3-Dimethoxyphenyl)2-dimethylthexysilyloxy-(1R,2S)-5-cyclohexen-1-yl-N-tert-(butoxycarbonyl)glycinate (196). Thick colorless oil; Rf = 0.4 ethyl acetate/ hexane 80:20; $[\alpha]_D^{25}$ –74.0 (c 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ: 6.9 (t, *J* = 7.9 Hz, 1H), 6.8 (dd, *J* = 8.2 Hz, 1H), 6.7 (dd, *J* = 7.6 Hz, 1H), 5.9 (bs, 2H), 4.9 (bs, 1H), 4.1 (m, 1H), 3.8 (s, 3H), 3.7 (s, 3H), 2.2 – 2.1 (m, 2H), 1.9 (m, 1H), 1.7 -1.6 (M, 1H), 1.6 - 1.5 (m, 1H), 1.4 (s, 9H), 0.9 (d, *J* = 6.7 Hz, 6H), 0.8 (d, *J* = 3.7 Hz, 6H), 0.1 (d, *J* = 11.9 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ: 168.4, 155.2, 154.1, 150.2, 134.6, 130.8, 130.6, 129.0, 128.9, 119.8, 110.3, 79.4, 71.7, 69.1, 54.3, 42.3, 34.2, 28.2, 25.0, 24.8, 24.4, 20.2, 20.1, 18.4, 18.3, -3.2; IR (KBr / cm^{-1}): 3443, 2931, 2105, 1643, 1470, 1366; HRMS: $C_{29}H_{48}NO_7Si$ (M+) Calcd. for $C_{29}H_{47}NO_7Si$: 550.5983. Found: 550.3197.

5.3.5. General Procedure for Claisen Rearrangement.

A solution of the glycine ester (1.0 eq) in THF (20 ml/mmol) and a 1.0 M solution of $ZnCl_2$ (1.2 eq.) in ether was cooled to -78ºC. Then a 2.0 M solution of LDA (2.4 eq.) in THF was added dropwise to the reaction mixture and the system was allowed to warm slowly (overnight) to room temperature. The basic solution was diluted with ethyl ether and quenched with 10% $KHSO_4$ solution in water to obtain an acidic solution (pH = 2. After extraction with ethyl ether (4 X 15 mL) and drying (magnesium sulfate), the solvent was removed to afford the crude rearranged amino acids as clear oils.(25-90%) This

compounds were used in the next step without further purification. A portion was treated with diazomethane to obtain the corresponding methyl esters and the rest of the material was used to prepare the lactones.

2-(4-Dimethylthexylsilyloxy-2-phenyl-(1*R*,4*S*)-2-cyclohexenyl)-(2*R*)-*N*-*tert*-butoxycarbonylmethylglycinate (199). Colorless oil; R_f = 0.7 hexanes:ethyl acetate, 80:20; $[\alpha]_D^{24}$ -5.74° (c 0.8, $CHCl_3$); IR ($CDCl_3$) 3620, 3463, 3022, 2975, 1731, 1708, 1499, 1438, 1368, 1251, 1224, 1162, 1049; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35 (m, 5H), 5.86 (bd, *J* = 2.0 Hz, 1H), 5.18 (d, *J* = 8.0 Hz, 1H), 4.40 (dd, *J* = 8.0, 3.0 Hz, 1H), 4.27 (m, 1H), 3.46 (s, 3H), 3.36 (bs, 1H), 1.86 (m, 2H), 1.72 (m, 1H), 1.60 (m, 2H), 1.43 (s, 9H), 0.91 (d, *J* = 7.0 Hz, 6H), 0.86 (s, 6H), 0.13 (s, 3H), 0.11 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 171.94, 155.04, 141.00, 139.41, 133.09, 128.28, 127.31, 126.87, 79.61, 65.77, 55.64, 51.64, 38.61, 34.31, 29.01, 28.36, 24.86, 22.06, 20.34, 18.64; LRMS (FAB) ***m/z*** (rel intensity) 503 (M^+, 2), 446 (18), 362 (32), 315, 288 (100); HRMS calcd for $C_{28}H_{45}NO_5Si$: 502.2988. Found: 502.2988. Anal. calcd for $C_{28}H_{45}NO_5Si$: C, 66.76; H, 9.00; N, 2.78. Found: C, 66.86; H, 9.07; N, 2.70.

2-(4-Dimethylthexylsilyloxy-2-phenyl-(1*R*,4*S*)-2-cyclohexenyl)-(2*S*)-*N*-*tert*-butoxycarbonylmethylglycinate (200). Colorless oil; R_f = 0.7 hexanes:ethyl acetate ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.28 (m, 5H), 6.00 (bd, *J* = 5.0 Hz, 1H), 4.85 (bd, *J* = 8.0 Hz, 1H), 4.29 (m, 1H), 4.18 (dd, *J* = 8.0, 5.0 Hz, 1H), 3.67 (s, 3H), 3.32 (s, 1H), 1.70(m, 5H), 1.36 (s, 9H), 0.94 (d, *J* = 7.0 Hz, 6H), 0.90(s, 3H), 0.89(s, 3H), 0.18(s, 3H), 0.15 (s, 3H)); ^{13}C NMR (75 MHz, $CDCl_3$) δ 140.44, 131.87, 128.40, 127.54, 126.52, 126.40, 79.35, 63.87, 54.57, 52.05, 38.23, 34.33, 29.67, 28.39, 28.26, 20.39, 18.74, 18.59, -2.25, -2.76.

Anal. calcd for $C_{28}H_{45}NO_5Si$: C, 66.76; H, 9.00; N, 2.78. Found: C, 66.86; H, 9.04; N, 2.76.

Note: Minor isomer. This compound was obtained with impurities of the major isomer and no further analysis was possible.

2-(4-Dimethylthexylsilyloxy-2-methyl-(1*R*,4*S*)-2-cyclohexenyl)-(2*R*)-*N*-*tert*-butoxycarbonylmethylglycinate (209). Colorless oil; R_f = 0.7 hexanes:ethyl acetate, 80:20; $[\alpha]_D^{24}$ -62.7° (c 1.0, $CHCl_3$); IR ($CDCl_3$) 3437, 2955, 2866, 1708, 1501, 1368, 1250, 1161, 1055; ^1H NMR (300 MHz, $CDCl_3$) δ 5.51 (bs, 1H), 5.16 (d, *J* = 8 Hz, 1H), 4.52 (dd, *J* = 8, 3 Hz, 1H), 4.06 (bd, *J* = 3 Hz, 1H), 3.67 (s, 3H), 2.58 (bs, 1H), 1.72 (s, 3H), 1.75-1.50 (m, 5H), 1.43 (s, 9H), 0.85 (d, *J* = 7Hz, 6H), 0.79 (s, 6H), 0.05 (s, 3H), 0.04 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.53, 155.37, 134.68, 129.33, 79.82, 65.25, 55.09, 52.02, 41.90, 34.27, 29.85, 28.35, 24.78, 21.78, 20.30, 18.60, -2.51, -2.79; Anal. calcd for $C_{23}H_{43}NO_5Si$: C, 62.55; H, 9.81; N, 3.17. Found: C, 62.73; H, 9.53; N, 3.14.

2-(4-Dimethylthexylsilyloxy-2-methyl-(1*R*,4*S*)-2-cyclohexenyl)-(2*S*)-*N*-*tert*-butoxycarbonylmethylglycinate (217). Colorless oil; R_f = 0.7 hexanes:ethyl acetate, 80:20; $[\alpha]_D^{24}$ -48.6° (c 1.0, $CHCl_3$); IR ($CDCl_3$) 3445, 2955, 2868, 1709, 1505, 1368, 1252, 1159; ^1H NMR (300 MHz, $CDCl_3$) δ 5.66 (bd, *J* = 4.0 Hz, 1H), 4.87 (d, *J* = 8.0 Hz, 1H), 4.56 (dd, *J* = 8.0, 4.0 Hz, 1H), 4.06 (bs, 1H), 3.72 (s, 3H), 2.60 (bs, 1H), 1.70(s, 3H), 1.50(m, 5H), 1.40 (s, 9H), 0.87 (d, *J* = 7.0 Hz, 6H), 0.81(s, 6H), 0.07 (s, 3H)), 0.05(s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.58, 155.75, 135.67, 129.16, 128.46, 127.34, 125.56, 79.66, 63.78, 54.15, 52.14, 40.15, 34.33, 30.27, 28.26, 24.84, 20.88, 20.38, 18.61, 18.35, -2.34, -2.89; LRMS (FAB) *m/z* (rel intensity) 440 (M^+-H, 7), 384 (14), 356 (16),

300 (49), 282 (100); HRMS calcd for $C_{23}H_{42}O_5NSi$ 440.2832. Found: 440.2853. Anal. calcd for $C_{23}H_{43}O_5NSi$ C, 62.55; H, 9.81; N, 3.17. Found: C, 62.57; H, 9.30; N, 3.09.

2-(2-chloro-4-dimethylthexylsilyloxy-(1*R*,4*S*)-2-cyclohexenyl)-(2*R*)-*N*-*tert*-butoxycarbonylmethylglycinate (210). Colorless oil; R_f = 0.7 hexanes:ethyl acetate, 80:20; $[\alpha]_D^{24}$ -63.0° (c 1.0, $CHCl_3$); IR ($CDCl_3$) 2956, 2868, 1711, 1501, 1368, 1252, 1163, 1092; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.95 (bd, *J* = 5.0 Hz, 1H), 5.14 (d, *J* = 9.0 Hz, 1H), 4.64 (dd, *J* = 9.0, 2.0 Hz, 1H), 4.19 (dd, *J* = 9.0, 4.0 Hz, 1H), 3.75 (s, 3H), 3.06 (bt, 7Hz 1H), 1.90(m, 1H), 1.81(m, 1H), 1.62 (m, 3H), 1.45 (s, 9H), 0.87 (d, *J* = 7.0 Hz, 6H), 0.81(s, 6H), 0.09 (s, 3H)), 0.07(s, 3H): ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.02, 155.61, 134.47, 131.94, 80.02, 65.59, 55.25, 52.36, 43.92, 34.20, 29.45, 28.29, 24.77, 23.01, 20.20, 18.56, -2.64, -2.90

2-(4-Dimethylthexylsilyloxy-2-(2-methoxyphenyl)-(1*R*,4*S*)-2-cyclohexenyl)-(2*R*)-*N*-*tert*-butoxycarbonylmethylglycinate (211). Colorless oil; R_f = 0.7 hexanes:ethyl acetate, 80:20; $[\alpha]_D^{24}$ -53° (c 0.7, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.24 (m, 1H), 7.01 (d, *J* = 7Hz, 1H), 6.88 (t, *J* = 10Hz, 2H), 5.78 (dd, *J* = 4.4, 2.7 Hz, 1H), 5.62 (d, *J* = 9.7 Hz, 1H), 4.23 (m, 2H), 3.84 (s, 3H), 3.38 (bt, 6Hz 1H), 3.21 (s, 3 H), 2.00 (m, 1H), 1.88 (m, 1H), 1.80-1.60 (m, 3H), 1.44 (s, 9H), 0.94 (dd, *J* = 6.6, 1.7 Hz, 6H), 0.87 (s, 6H), 0.15 (s, 3H), 0.13 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.02, 156.37, 156.04, 135.96, 134.85, 130.22, 129.69, 128.63, 120.15, 110.69, 79.13, 64.68, 56.63, 55.12, 51.61, 40.28, 34.48, 29.80, 28.36, 24.81, 23.57, 20.44, 20.35, 18.71, -2.46, -2.78.

2-(4-Dimethylthexylsilyloxy-2-(2-methoxyphenyl)-(1*R*,4*S*)-2-cyclohexenyl)-(2*S*)-*N*-*tert*-butoxycarbonylmethylglycinate (219). Colorless oil; R_f = 0.7 hexanes:ethyl acetate, 80:20; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.24 (m, 1H), 7.01 (d, *J* = 7.4 Hz, 1H), 6.86 (t, *J* = 7.4 Hz, 2H), 5.90 (bd, *J* = 4.9, Hz, 1H), 4.80 (d, *J* = 7.4 Hz, 1H), 4.25 (bt, *J* = 2, 1H), 3.95 (dd, *J* = 7.4, 4.1 Hz, 1H), 3.83 (s, 3H), 3.67 (s, 3 H), 3.56 (bs, 1H), 1.80-1.60 (m, 4H), 1.50(m, 1H), 1.36 (s, 9H), 0.94 (dd, *J* = 6.6, 1.7 Hz, 6H), 0.87 (s, 6H), 0.14 (s, 3H), 0.12 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.71, 156.52, 155.10, 140.06, 132.33, 130.21, 129.37, 128.81, 120.89, 110.45, 79.17, 63.50, 55.38, 54.78, 52.01, 38.36, 34.45, 30.14, 28.30, 24.87, 20.41, 18.68, 18.21, -2.32, -2.81.

Note: Minor isomer. This compound was obtained with impurities of the major isomer and no further analysis was possible.

5.3.6. General Procedure for Lactonization.

The crude epimeric mixture of amino acids were stirred with *p*-TsOH in Dichloromethane at room temperature for 5 hours. The reaction mixture was diluted with ethyl ether and washed with aqueous sodium bicarbonate (2 X 10 mL) and brine (2 X 5 mL). The organic layer was dried with anhydrous magnesium sulfate, and the solvent was evaporated under reduced pressure to obtain the epimeric mixture of lactones **8** and **9**, which were separated by column chromatography (hexane/ethyl acetate 80:20) to afford the individual compounds (50-60%).

3-*tert*-Butoxycarbonylamino-7a-phenyl-(3*R*,3a*S*,7a*S*)-2,3,3a,4,5,7,7a-hexahydroben-zo[b]furan-2-one (203). White crystalline solid; R_f = 0.5 hexanes/ethyl acetate, 80:20; $[\alpha]_D^{23}$ -50.3° (c 0.8, $CHCl_3$); IR ($CHCl_3$) 2980, 2931, 2873, 1773, 1715, 1506, 1258, 1161 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.33 (m, 5H), 6.20 (m, 1H), 5.76

(ddd, J = 10, 2 1 Hz, 1H), 5.03 (bd, J = 6 Hz, 1H), 4.53 (dd, J = 7, 6 Hz, 1H), 3.10 (ddd, J = 13, 7, 5 Hz, 1H), 2.28 (m, 2H), 1.86 (m, 1H), 1.42 (s, 9H), 1.24 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.41, 155.36, 141.78, 132.73, 128.72, 127.97, 127.87, 124.68, 83.58, 80.42, 53.91, 45.08, 28.18, 22.93, 20.08 LRMS (Cl CH_4) *m/z* (rel intensity) 659 ($2M^+$+1, 54) 330 (M^++1, 100), 274 (30), 228 (20), 136; HRMS calcd for $C_{19}H_{24}NO_4$: 330.1705. Found: 330.1733.

3-*tert*-Butoxycarbonylamino-7a-phenyl-(3*S*,3a*S*,7a*S*)-2,3,3a,4,5,7,7a-hexahydroben-zo[b]furan-2-one (204). White crystalline solid; R_f = 0.5 hexanes/ethyl acetate, 80:20; $[\alpha]_D^{23}$ -13.9° (c 0.4, $CHCl_3$); IR ($CHCl_3$) 3026, 2930, 1777, 1711, 1502, 1298, 1210, 1163 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 (m, 5H), 6.20 (m, 1H), 5.78 (bd, J = 10, Hz, 1H), 4.97 (bs, 1H), 4.59 (m, 1H), 2.52 (m, 1H), 2.46 (m, 1H), 2.24 (m, 1H), 1.90 (m, 2H), 1.42 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 132.00, 128.41, 128.21, 125.95, 125.67, 52.30, 50.00, 28.24, 19.93, 18.30.

Note: Minor isomer. This compound was obtained with impurities of the major isomer and no further analysis was possible.

3-*tert*-Butoxycarbonylamino-7a-methyl-(3*R*,3a*S*,7a*S*)-2,3,3a,4,5,7,7a-hexahydroben-zo[b]furan-2-one (221). White crystalline solid; R_f = 0.5 hexanes/ethyl acetate, 80:20; $[\alpha]_D^{23}$-31.4° (c 0.7, $CHCl_3$); IR ($CHCl_3$) 3025, 2982, 2932, 1770, 1712, 1506, 1369, 1302, 1215, 1163 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.00 (dd, J = 9, 7 Hz, 1H), 5.76 (ddd, J = 10, 3, 1 Hz, 1H), 4.97 (bs, 1H), 4.86 (dd, J = 7, 6 Hz, 1H), 2.64 (ddd, J = 13, 7, 5 Hz, 1H), 2.16 (m, 1H), 1.99 (m, 1H), 1.70 (m, 1H), 1.49 (s, 3H), 1.46 (s, 9H), 1.03 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 132.76, 128.02, 80.51, 54.64, 43.18, 28.29, 26.33, 22.86, 19.90; LRMS (CI) *m/z* (rel intensity) 268 (M^++1, 100), 212 (51), 166 (31),

122(19); HRMS calcd for $C_{14}H_{22}NO_4$: 268.1549. Found: 268.1541; Anal. calcd for $C_{14}H_{21}NO_4$: C, 62.90; H, 7.92; N, 5.24. Found: C, 62.79; H, 7.97; N, 5.34.

3-*tert*-Butoxycarbonylamino-7a-methyl-(3*S*,3a*S*,7a*S*)-2,3,3a,4,5,7,7a-hexahydroben-zo[b]furan-2-one (224). White crystalline solid; R_f = 0.5 hexanes/ethyl acetate, 80:20; $[\alpha]_D^{23}$ -22.0° (c1.0, $CHCl_3$); IR ($CHCl_3$) 3020, 2982, 2933, 1765, 1710, 1502, 1369, 1322, 1163 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 5.86 (dd, *J* = 9, 3 Hz, 1H), 5.62 (d, *J* = 10 Hz, 1H), 4.96 (bd, *J* = 7 Hz, 1H), 4.48 (m, 1H), 2.40 (m, 1H), 2.32 (m, 1H), 2.06 (m, 2H), 1.84 (m, 1H), 1.50 (s, 3H), 1.44 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 129.96, 128.01, 81.5, 80.5, 51.98, 47.63, 28.24, 26.21, 19.99, 19.51; Anal. calcd for $C_{14}H_{21}NO_4$: C, 62.90; H, 7.92; N, 5.24. Found: C, 62.83; H, 7.85; N, 5.35.

3-*tert*-Butoxycarbonylamino-7a-(2-methoxyphenyl)-(3*R*,3a*S*,7a*S*)-2,3,3a,4,5,7,7a-hexahydrobenzo[b]furan-2-one (222). Clear oil; R_f = 0.5 hexanes/ethyl acetate, 80:20; ^1H NMR (300 MHz, $CDCl_3$) δ 7.39 (dd, *J* = 7.7, 1.4 Hz, 1H), 7.29 (dt, *J* = 8.0, 1.7 Hz, 1H), 6.92 (m, 2H), 6.14 (m, 1H), 5.62 (dt, *J* = 9.9, 1.4 Hz, 1H), 4.98 (bd, *J* = 5.2 Hz, 1H), 4.47 (dd, *J* = 5.8, 1.4 Hz, 1H), 3.78 (s, 3H), 3.43 (ddd, *J* = 13.5, 7.4, 4.9 Hz, 1H), 2.20 (m, 2H), 1.81 (m, 1H), 1.42 (s, 9H), 1.28-1.07 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.84, 155.76, 132.36, 129.51, 129.17, 126.11, 125.35, 120.71, 111.44, 82.93, 80.26, 55.30, 54.27, 41.50, 28.21, 22.75, 20.65.

3-*tert*-Butoxycarbonylamino-7a-(2-methoxyphenyl)-(3*S*,3a*S*,7a*S*)-2,3,3a,4,5,7,7a-hexahydrobenzo[b]furan-2-one (226). Clear oil; R_f = 0.5 hexanes/ethyl acetate, 80:20; ^1H NMR (300 MHz, $CDCl_3$) δ 7.41-7.26 (m, 2H), 7. (m, 2H), 6.19 (m, 1H), 5.89 (dt, *J* = 10.3, 1.0 Hz, 1H), 4.99 (bd, *J* = 6.6 Hz, 1H), 4.64 (dd, *J* = 8, 3 Hz, 1H), 3.84 (s, 3H), 3.04 (dtd, *J* = 11.5, 3.5, 1 Hz, 1H), 2.26-2.10 (m, 1H), 2.01-1.89 (m, 1H),

1.72-1.58 (m, 2H),1.44 (s, 9H), ^{13}C NMR (75 MHz, $CDCl_3$) δ 174.84, 157.89, 131.84, 130.25, 129.16, 128.02, 126.12, 120.08, 112.57, 83.50, 80.31, 56.04, 51.70, 45.37, 28.25, 20.11, 19.18;

Note: Minor isomer. This compound was obtained with impurities of the major isomer and no further analysis was possible.

3-tert-Butoxycarbonylamino-7a-(2,3-dimethoxyphenyl)-3*S*,3a*S*,7a*S*)-2,3,3a,4,5,7,7a-hexahydro[b]furan-2-one (223). White crystals; Rf = 0.5 hexane: ethyl acetate, 80:20 $[\alpha]_D^{32}$ - 96.0 (c 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.1 -6.9 (m, 2H), 6.8 -6.7 (m, 2H), 6.2 (m, 1H), 5.7 (dt, *J* = 10.0, 1.0 Hz, 1H), 4.9 (d, *J* = 5.7 Hz, 1H), 4.5 (dd, *J* = 7.9, 3.0 Hz, 1H), 3.8 (s, 3H), 3.7 (s, 3H), 3.3 (dtd, *J* = 11.5, 3.5, 1.0 Hz, 1H), 2.3 -2.2 (bm, 2H), 1.7 - 1.6 (m, 1H), 1.4 (s, 1H), 1.3 (s, 9H); ^{13}C NMR ($CDCl_3$) δ: 174.9, 155.3, 153.4, 135.1, 132.5, 126.9, 123.6, 117.2, 112.9, 82.8, 80.3, 59.9, 55.8, 54.1, 42.9, 29.7, 28.2, 22.8, 20.5; IR (KBr/ cm^{-1}): 2932, 2253, 1776, 1716, 1506, 1475, 1263; LRMS (Cl/ CH_4) *m/z* (rel. intensity) 389 (m^+, 70), 334 (65), 228 (100); HRMS Calcd. for $C_{22}H_{36}NO_6$ (m+1) Calcd.: 389.2464; Found: 389.5326. Anal Calcd. for $C_{23}H_{35}NO_6$: C, 64.70; H, 6.90; Found: C, 64.36; H, 6.64.

5.3.7. General Procedure for Isomerization of Lactones.

Lactones (0.03 mmoles) were dissolved in THF (1mL) and DBU (4.6mg, 0.03mmoles) was added at room temperature. The reaction mixture was stirred under Argon and the isomerization was monitored by TLC (hexanes:ethyl acetate 80:20) and HPLC (MeCN:H_2O 60/40).

5.4. Synthesis of Narciclasine

4,6-dibromo-2,2-dimethyl-(3a*S*,7a*S*)-benzo[d](1,3)-dioxole (169). Diol **167** (0.2 mmol) was dissolved in acetone (2 mL) and DMP (2 mL) was added followed by TsOH (cat.) and the reaction mixture was stirred at room temperature for 5 to 30 minutes. solid K_2CO_3 (excess) was added and the solids were filtered. The solvent was removed under reduced pressure and the resulting oil was used in the next step without purification. Analytical samples were purified by flash column chromatography using hexane/ethyl acetate mixtures and used immediately in the next step.

Yield: 100%. Yellow oil (unstable compound); Rf = 0.5 (hexanes \ ethyl acetate : 80/20); $[\alpha]_D^{26}$ = +23.3 (C = 1.0, EtOH); ^{1}H NMR ($CDCl_3$, 300 MHz) 6.42 (dd, *J* = 1.1, 0.8 Hz, 1H), 6.25 (ddd, *J* = 4.1, 2.5, 1.1 Hz, 1H), 4.68 (m, 2H), 1.42 (s, 3H), 1.39 (s, 3H); ^{13}C NMR ($CDCl_3$, 300 MHz) 129.7, 126.1, 124.1, 118.0, 107.1, 74.7, 73.5, 26.7, 25.0.

1,8-dibromo-11-carbomethyloxy-4,4-dimethyl-(1*R*,2*S*,6*S*,7*S*)-3,5,10,11-trioxaazatricyclo[5.2.2.0^{2,6}]-8-undecene (171). To a solution of the diol **167** (1 mmol) in dimethoxypropane (13 mL) was added a catalytic amount of *p*-toluenesulfonic acid. When no more starting material remained, the solution was cooled to 0 °C, and water (1 mL) was added. Sodium periodate (1 mmol) was carefully added to the reaction vessel, and the carbamate (1 mmol) was dissolved in 1 mL of methanol, and introduced dropwise to the mixture. The reaction mixture was warmed to room temperature, and allowed to react overnight. Upon completion, aqueous sodium bisulfite was added, and the product was extracted with diethyl ether (3 X 50 mL). The organic phase was washed with brine (2 X 10 mL), dried with magnesium sulfate, and concentrated. The final product was

isolated via column chromatography using a 7/3 mixture of hexanes/ethyl acetate as eluent.

Yield: 70%. Brownish solid; mp: 150-152 °C; Rf = 0.3 (hexanes \ ethyl acetate : 70/30); $[\alpha]_D^{25}$ = + 36.4 (C = 1.1, $CHCl_3$); IR (KBr): 4214, 3684, 3619, 3019, 2400, 1522, 1423, 1214; ^1H NMR ($CDCl_3$, 300 MHz) 6.70 (d, 0.9 Hz 1H), 5.17 (dd, *J* = 3.9, 2.1 Hz, 1H), 4.70 (dd, *J* = 6.9, 4.5 Hz, 1H), 4.56 (d, *J* = 7.0 Hz, 1H), 3.84 (s, 3H), 1.43 (s, 3H), 1.36 (s, 3H); ^{13}C NMR ($CDCl_3$, 300 MHz) 157.8, 132.6, 120.7, 112.1, 87.0, 81.0, 74.5, 61.2, 54.3, 25.7, 25.4; MS (FAB): 401 (^{81}Br+^{81}Br, M^+), 400 (^{81}Br+^{80}Br, M^+), 399 (^{80}Br+^{80}Br, M^+); HRMS: calcd for $C_{11}H_{14}NBr_2O_5$: 399.9219; Found: 399.9195; Anal calcd. for $C_{11}H_{14}NBr_2O_5$: C 33.11, H 3.28, N 3.51. Found: C 33.23, H 3.29, N 3.43.

1-bromo-11-carbomethyloxy-4,4-dimethyl-8-(7-methoxybenzo[d][1,3]dioxol-5-yl)-(1*R*,2*S*,6*S*,7*S*)-3,5,10,11-trioxaazatricyclo[5.2.2.0^{2,6}]-8-undecene (240). An argon-flushed 25 mL flask was charged with $Pd(PPh_3)_4$ (0.05 mmol), benzene (10 mL), dibromooxazine **171** (1 mmol), and 2M aq. Na_2CO_3 (2 mL). The borate (1.2 mmol) was then added as a solution in ethanol, and the mixture was refluxed overnight. The product was extracted with diethyl ether (3 X 25 mL), and washed with 5% HCl (1 X 10 mL) and brine (3 X 10 mL). The extract was dried ($MgSO_4$), filtered and concentrated. The residue was purified via column chromatography with a gradient of hexanes and ethyl acetate as eluent.

Yield: 30%. m.p.= 158-160 °C; $[\alpha]_D^{25}$ = 37.1 (C = 1.0, $CHCl_3$); Rf = 0.7 (hexanes \ ethyl acetate : 70/30); ^1H NMR ($CDCl_3$, 300 MHz) 6.73 (d, *J* = 1.6 Hz, 1 H), 6.68 (d, *J* = 1.6 Hz, 1 H), 6.49 (dd, *J* = 2.2, Hz, 1 H), 5.97 (s, 2 H), 5.38 (dd, *J* = 4.1, 2.1 Hz, 1 H), 4.72 (dd, *J* = 7.0, 4.1 Hz, 1 H), 4.65 (dd, *J* = 6.9, 1.0, 1 H), 3.91 (s, 3 H), 3.73 (s, 3 H),

1.32 (s, 3 H), 1.26 (s, 3 H); ^{13}C NMR ($CDCl_3$, 300 MHz) 158.2, 149.4, 143.8, 143.7, 136.4, 129.7, 124.0, 111.7, 106.1, 101.8, 100.3, 87.8, 81.2, 74.2, 56.6, 55.9, 54.1, 25.8, 25.3; IR (KBr): 4214, 3019, 2400, 1214; MS: 472 (^{81}Br M^+ + H), 470 (^{79}Br M^+ + H), 332, 290, 154; HRMS: Calcd for $C_{19}H_{21}N^{81}BrO_8$: 472.0434. Found: 472.0423; Calcd for $C_{19}H_{21}N^{79}BrO_8$: 470.0451. Found: 470.0360; Anal. Calcd: C 48.53, H 4.29, N 2.98. Found: C 48.52, H 4.33, N 2.90

7-aminocarbomethoxy-2,2-dimethyl-6-(7-methoxybenzo[d][1,3]dioxol-5-yl)-(3aS,7R,7aS)-4,7-dihydrobenzo[d][1,3]dioxol-4-one (241). Oxazine **240** (1 mmol) was dissolved in benzene (10 mL). The solution was degassed and TTMS (1.1 mmol) was added. The mixture was heated to reflux and AIBN (cat.) was added. Heating and stirring was continued for 45 to 90 minutes and then the reaction mixture was allowed to cool down to room temperature. The solvent was removed under reduced pressure and the residue purified by flash column chromatography using a gradient of hexane and ethyl acetate as eluent.

Yield: 80%. mp: 81- 84 °C; Rf = 0.5 (ethyl acetate : 100); $[\alpha]_D^{26}$ = -26.8 (c = 1.1 $CHCl_3$); IR (neat) 3006, 2806, 2706, 1686, 1184; ^1H NMR ($CDCl_3$, 300 MHz) 6.83 (s, 1H), 6.74 (d, J = 1.2 Hz, 1H), 6.38 (s, 1H), 5.99 (s, 2H), 5.47 (d, 8.8 Hz, 1H), 5.24 (d, J = 8.0 Hz, 1H), 4.63 (dd, J = 5.0, 2.2 Hz, 1H), 4.42 (d, J = 5.1 Hz, 1H), 3.87 (s, 3H), 3.65 (s, 3H), 1.37 (s, 3H), 1.28 (s, 3H); ^{13}C NMR ($CDCl_3$, 300 MHz) 195.7, 156.2, 153.2, 149.6, 143.8, 138.0, 129.9, 123.6, 110.4, 107.5, 102.2, 100.9, 77.1, 73.4, 56.6, 52.7, 47.9, 27.4, 25.9; MS (CI): 392 ($(M+H)^+$, 100), 391 (M^+, 92), 291 (18), 187 (7); HRMS Calcd for $C_{19}H_{22}NO_8$: 392.1345. Found: 392.1320 Anal. Calc. C 58.31, H 5.41, N 3.58; Found: C 68.61, H 5.56, N 3.29

Tandem Suzuki coupling – oxazine reduction. An Argon-flushed 25 mL flask was charged with $Pd(PPh_3)_4$ (0.05 mmol), Benzene (10 mL), dibromooxazine **171** (1 mmol), and 2M aq. Na_2CO_3 (2 mL). The borate (1.2 mmol) was then added as a solution in ethanol, and the mixture was refluxed for 8 hours. Then acetonitrile (5 mL) and molybdenum hexacarbonyl (1 mmol) were added. The dark heterogeneous solution was heated to reflux for 10 hours or until the coupled oxazine **240** was consumed. The reaction mixture was cooled and filtered through silica gel. The filtrate was concentrated and pumped for 1 hour. The solid residue was dissolved in ethyl acetate and the insoluble material removed through another filtration. The new filtrate was concentrated and the residue purified by column chromatography using a gradient of hexanes and ethyl acetate as eluent. This procedure yield ketone **241** in 45% from **171** without isolation of intermediate oxazine **240** (all data is presented above).

7-aminocarbomethyloxy-2,2-dimethyl-6-(7-methoxybenzo[d][1,3]dioxol-5-yl)-(3aR,4R,7R,7aS)-4,7-dihydrobenzo[d][1,3]dioxol-4-ol (244). To a solution of ketone **241** (1 mmol) in methanol (6.5 mL) at room temperature, was added cerium chloride (1.5 mmol). The mixture was stirred for 5 minutes and cooled to 0 °C, then sodium borohydride (1.1 mmol) was added. The ensuing mixture was stirred at 0 °C for 30 minutes. Upon completion (TLC), the reaction was quenched by adding a few drops of 50% acetic acid. Water (10 mL) and methylene chloride (10 mL) were added and the heterogeneous mixture was extracted with methylene chloride (4 X 20 mL). The organic layer was washed with water (3 X 10 mL), and brine (2 X 5 mL) to remove the methanol. The extract was dried with magnesium sulfate, and concentrated. The residue was purified by column chromatography using pure ethyl acetate as the eluent.

Yield: 80%. White solid mp: 91 - 94 °C. Rf = 0.2 (ethyl acetate : 100); $[\alpha]_D^{25}$ = -14.4 (c = 0.8, $CHCl_3$); IR (KBr) 3006, 2820, 2660, 1460, 1381; ^{1}H-NMR ($CDCl_3$, 300MHz) 6.54 (s, 1H), 6.53 (s, 1H), 6.05 (s, 1H), 5.92 (s, 2H), 4.65 (m, 4H), 4.40 (d, *J* = 9 Hz, 1H), 3.85 (s, 3H), 3.64 (s, 3H), 2.88 (d, *J* = 10 Hz, 1H), 1.30 (s, 3H), 1.27 (s, 3H); ^{13}C NMR ($CDCl_3$, 300MHz) 156.5, 149.1, 143.5, 137.0, 135.2, 133.6, 130.6, 109.2, 105.6, 101.5, 99.8, 66.5, 56.8, 52.3, 51.0, 26.1, 24.5; MS (FAB) 392(M^+-H), 334, 259, 173; HRMS Calcd for $C_{19}H_{22}NO_8$: 392.1345. Found: 392.1340. Anal. Calcd: C 58.01, H 5.89. Found: C 58.07, H 6.29

7-aminocarbomethyloxy-2,2-dimethyl-6-(7-methoxybenzo[d][1,3]dioxol-5-yl)-(3aR,4S,7R,7aS)-4,7-dihydrobenzo[d][1,3]dioxol-4-yl benzoate (245). To a solution of alcohol **12** (1 mol) in anhydrous THF, was added tributyl phosphine (2 mol), benzoic acid (2 mol) under argon, keeping the temperature at 25 °C. DEAD (2 mol) was then added dropwise, and the solution was stirred for 4 hours under argon at room temperature. After the reaction was complete, the mixture was concentrated, and purified via column chromatography using a mixture of hexanes and ethyl acetate as eluent.

Yield: 65%. Rf = 0.5 (60:40 hexanes/ethyl acetate) $[\alpha]_D^{25}$ = -12.22 (C = 1.0, $CHCl_3$); IR ($CHCl_3$): 3420, 3000, 1745, 1720, 1492, 1240, 1096, 1058; ^{1}H NMR (C_6D_6, 300 MHz) 8.08 (d, *J* = 6.6 Hz, 2H), 7.75 (d, *J* = 5.5 Hz, 1H), 6.89 (s, 1H), 6.80 (d, *J* = 1.1 Hz, 1H), 6.28 (d, *J* = 6.6 Hz, 1H), 5.85 (dd, *J* = 6.6, 1.4 Hz, 1H), 5.40 (s, 2H), 5.28 (s, 2H), 4.58 (d, *J* = 6.9 Hz, 1H), 4.37 (d, *J* = 6.9 Hz, 1H), 3.50 (s, 3H), 3.40 (s, 3H), 1.29 (s, 3H), 1.13 (s,3H). ^{13}C NMR (C_6D_6, 300 MHz) 165.0, 156.2, 149.9, 145.4, 144.3, 133.6, 133.4, 131.6, 129.9, 121.3, 108.7, 107.1, 101.43, 100.4, 78.0, 74.9, 69.0, 63.5, 62.4, 56.3, 52.1, 50.5, 26.5, 24.4, 14.23, 13.62; MS (CI): 497 (M^+), 480, 376, 318, 281, 215, 105.

HRMS Calcd for $C_{26}H_{27}NO_9$ (M^+): 497.1686, Found: 497.1716; $C_{26}H_{28}NO_9$ ($M+H^+$): 498.1764, Found: 498.1752.

6-aminocarbomethoxy-1,2-dihydroxy-5-(7-methoxybenzo-[d][1,3]dioxol-5-yl)-4-cyclohexene-1-yl benzoate (247). Benzoate **245** (1 mmol) was dissolved in methanol and a spatula tip (approx. 1/5 w/w) of Dowex 50X8-100 ion exchange resin was added. The mixture was stirred overnight at room temperature, then the resin was separated by filtration and the solvent removed under reduced pressure to afford the corresponding diol.

The crude was dissolved in pyridine (15 mmol) and cooled to 0°C. Acetic anhydride (10 mmol) and DMAP (cat.) were added and the reaction mixture stirred for 3 hours at room temperature. Ether (20 mL) and water (2 mL) were added and the mixture transferred to a separation funnel. The organic layer was washed several times with 2 mL aliquots of 10% aq. $CuSO_4$ until all the pyridine was removed and one more time with 2 mL of brine. The solution was dried with magnesium sulfate and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography using hexane-ethyl acetate mixtures.

Yield: 70%. White solid mp: 112-115 °C; $[\alpha]_D^{26}$ = -11.5 (c = 1.0, $CHCl_3$); IR (KBr): 3368 (br), 2903, 1750, 1720, 1602, 1521, 1447, 1379, 1223, 988, 807; ^1H NMR ($CDCl_3$, 300 MHz) 8.40 (dd, *J* = 8.5, 1.7 Hz, 2H), 7.57 (tt, *J* = 7.4, 1.4 Hz, 1H), 7.44 (t, *J* = 7.7 Hz, 2H), 6.58 (s, 1H), 6.57 (s, 1H), 6.10 (d, *J* = 3.0 Hz, 1H), 5.94 (s, 2H), 5.82 (dd, *J* = 6.9, 3.0 Hz, 2H), 5.57 (dd, *J* = 4.4, 2.5 Hz, 1H), 5.49 (dd, *J* = 7.1, 2.5 Hz, 1H), 4.90 (bs, 1H), 4.80 (d, *J* = 8.8 Hz, 1H), 3.86 (s, 3H), 3.64 (s, 3H), 2.12 (s, 3H), 2.03 (s,3H); ^{13}C NMR ($CDCl_3$, 300 MHz) 170.2, 169.9, 165.9, 149.2, 143.5, 133.4, 131.2, 129.8,

129.8, 129.5, 128.5, 128.5, 123.2, 106.2, 101.7, 100.6, 70.9, 69.6, 69.0, 56.6, 52.6, 51.0, 20.9, 20.8 (3 quaternary carbons below noise level); MS (FAB) 541 (M^+, 2), 391 (60), 149 (100); HRMS: Calcd for $C_{27}H_{27}NO_{11}$: 541.1584, Found: 541.1627

Partial data for the intermediate diol that was not purificated.

6-aminocarbomethoxy-1,2-dihydroxy-5-(7-methoxybenzo-[d][1,3]dioxol-5-yl)-4-cyclohexene-1-yl benzoate (246). ^{1}H NMR ($CDCl_3$, 300 MHz) 8.04 (d, J = 8 Hz, 2 H), 7.75 (t, J = 8 Hz, 1 H), 7.41 (t, J = 8 Hz, 2H), 6.58 (s, 1 H), 6.56 (s, 1 H), 6.04 (d, J = 2 Hz, 1 H), 5.93 (s, 2 H), 5.75 (dd, J = 4, 7 Hz, 1 H), 4.85 (s, 2 H), 4.20 (s, 1 H), 4.13 (s, 1 H), 3.84 (s, 3 H), 3.60 (s, 3 H), 2.02 (s, 1 H).

3,4-diacetoxy-7-methoxy-(2*S*,3*R*,4*S*,4a*R*)-2,3,4,6-tetrahydro[1,3]dioxolo[4,5-j]phenanthridin-6-one-2-yl benzoate (248). Diacetate **247** (1 mmol) was dissolved in dichloromethane (20 mL) and DMAP (3 mmol) was added. The solution was cooled to -10°C (acetone-ice bath), and triflic anhydride (5 mmol) was injected. The reaction mixture was stirred at -10 °C to -5 °C for 5 hours and overnight at 0°C. THF (5 mL) was added to allow for TLC monitoring. After verifying complete reaction of the starting material the solvents were removed under reduced pressure and THF (10 ml) was added. The solution was cooled to 0 °C and two drops of 2N HCl were added. The mixture was stirred for two hours and solid $NaHCO_3$ was added. The solvent was removed under reduced pressure and the residue purified by flash column chromatography using hexane-ethyl acetate mixtures.

Yield: 40%. Yellow oil; Rf = 0.1 (2:1 hexanes/ethyl acetate); $[\alpha]_D^{26}$ = + 22.4 (c = 1.1, $CHCl_3$); IR($CHCl_3$) 3490 (br), 2940, 2858, 1660, 1613, 1265, 1175, 1114; ^{1}H NMR ($CDCl_3$, 300 MHz): 8.04 (dd, J = 2, 9 Hz, 2H), 7.56 (tt, J = 2, 4, 8, Hz, 1H), 7.43 (t, J = 8

Hz, 2H), 6.79 (s, 1H), 6.25 (m, 1H), 6.05 (d, J = 2 Hz, 1H), 6.00 (d, J = 2 Hz, 1H), 5.94 (s, 1H), 5.57 (m, 2H), 5.35 (dd, J = 2, 9 Hz, 1H), 4.57 (bd, J = 10 Hz, 1H), 4.04 (s, 3H), 2.12 (s, 3H), 2.09 (s, 3H); ^{1}H NMR ($CDCl_3$, 300 MHz): 133.6, 129.9, 128.6, 117.6, 102.0, 99.6, 71.6, 68.89, 68.2, 61.0, 50.0, 20.9, 20.8; IR: 3490 (br), 2940, 2858, 1660, 1613, 1265, 1175, 1114; MS (FAB): 510, 391, 173, 163; HRMS: Calcd for $C_{26}H_{24}NO_{10}$: 510.1400. Found: 510.1419.

2,3,4-Trihydroxy-7-methoxy-(2*S*,3*R*,4*S*,4a*R*)-2,3,4,6-tetrahydro[1,3]dioxolo[4,5-j]phenanthridin-6-one (7-*O*-methylnarciclasine) (249). Phenanthridone **248** (1 mmol) was dissolved in methanol (10 mL) and Amberlyst A-21 ion exchange resin was added (approx. 1/1 w/w). The mixture was stirred for 2 hours at room temperature until all the starting material and a transient intermediate (prob. a diol) were consumed. The resin was separated by filtration and the solvent removed under reduced pressure.

Note: This compound is a known derivative of narciclasine[28]

Yield: 80%. White solid; Rf = 0.4 (CH_2Cl_2/MeOH : 80/20; IR (KBr) 3423 (br.), 2952, 2366, 1631 (amide), 1465, 1380, 1094, 825; $[\alpha]_D^{26}$ = 204 (c = 0.3, DMSO) ^{1}H NMR (CD_3OD, 300 MHz) 6.91 (s, 1H), 6.17 (m, 1H), 6.80 (d, J = 1.1 Hz, 1H), 6.02 (d, J = 1.1 Hz, 1H), 4.24 (m, 2H), 3.98 (s, 3H), 3.90 (m, 2H); ^{13}C NMR (CD_3OD, 300 MHz) 154.1, 145.5, 140.2, 135.2, 133.7, 123.7, 115.0, 103.6, 100.5, 74.2, 71.0, 70.8, 61.1, 53.6; MS (LC/ESI MS): 653.5 ([M+H+M]$^+$), 322 ([M+H]$^+$)

2,3,4,7-tetrahydroxy-(2*S*,3*R*,4*S*,4a*R*)-2,3,4,6-tetrahydro[1,3]dioxolo[4,5-j]phenanthridin-6-one (narciclasine) (2). The crude triol **249** (15 mG) was dissolved in anhydrous *N*,*N*-dimethylformamide and anhydrous LiCl (aprox. 10 mG) were added

under a stream of argon. The mixture was heated to 120 °C for 4 hours when the starting material was consumed. The solvent was removed under vacuum and the residue was adsorbed on silica gel and purified by column chromatography using dichloromethane/methanol: 80/20 as eluent. The procedure was repeated twice and a pure sample of narciclasine was obtained.

Narciclasine data matched the reported data for the natural alkaloid[29] as well as the synthetically prepared by Rigby.[21]

Yield: 10-20%. Rf = 0.6 (CH_2Cl_2/MeOH : 80/20; $[\alpha]_D^{23}$ 130 (c = 0.03 in DMSO); ^{1}H NMR (d^6-DMSO, 500 MHz) 13.25 (s, 1H), 7.88 (s, 1H), 6.85 (s, 1H), 6.15 (dd, J = 4.5, 2.8 Hz, 1H), 6.08 (m, 2H), 5.19 (d, J = 6.3 Hz, 1H), 5.16 (d, J = 5.6 Hz, 1H), 5.01 (d, J = 3.8 Hz, 1H), 4.18 (ddd, J = 8.6, 2.4, 1.4 Hz, 1H), 4.01 (m, 1H), 3.79 (ddd, J = 8.0, 5.5, 2.2 Hz, 1H), 3.69 (m, 1H).

Signals at 13.25, 7.88, 5.19, 5.16, and 5.01 can exchange deuterium on addition of deuterium oxide.

6-bromo-4-methoxybenzo[d][1,3]dioxole (152). Prepared in four steps from *o*-vanillin according to published procedures[115,116]

^{1}H-NMR ($CDCl_3$, 300 MHz) 6.67 (s, 2H), 5.97 (s, 2H), 3.90 (s, 3H); ^{13}C NMR ($CDCl_3$, 300 MHz) 127.3, 56.9, 106.2, 101.8, 113.3, 111.4, 111.3, 111.2; HRMS Calcd for $C_8H_7BrO_3$: 229.9579 Found: 229.9591; Anal Calcd for $C_8H_7BrO_3$: C 41.59, H 3.05 Found: C 41.57, H 3.00

4-methoxybenzo[d][1,3]dioxole-6-boronic acid (236). A solution of **152** (10 mmol) in anh. tetrahydrofuran (30 mL) was submerged in a dry ice-acetone bath and *t*-BuLi (0.7M in hexane, 12 mmol) was added dropwise. A dark purple color was

developed. The mixture was allowed to react for 15 minutes and then triethylborate (14 mmol) was injected dropwise. The red color vanished 5 minutes after the addition was complete. The reaction was allowed to react for 2 hours inside the cold bath and then quenched with saturated aqueous ammonium chloride solution. Ethyl acetate (30 mL) and water (20 mL) were added and the aqueous phase extracted with ethyl acetate (4 X 20 mL). The extract was washed with brine (2 X 10 mL), dried ($MgSO_4$) and concentrated, to render borate **2** as a white-gray solid. The compound did not tolerate chromatographic purification but was pure enough to proceed to the coupling step.

Note: The compound did not tolerate chromatographic purification.

Yield 70%. Yellowish solid (unstable) mp: >200 °C; ^{1}H-NMR (CD_3OD, 300 MHz) 6.96 (s, 1H), 6.81 (s, 1H), 5.82 (s, 2H), 3.79 (s, 3H); ^{13}C NMR (CD_3OD, 300 MHz) 115.4, 108.15, 103.12, 103,12, 103,12, 102.30, 57.25

5.5. Miscellaneous Compounds

2-hydroxy-3-methoxybromobenzene (bromoguaicol) (147). In a flame-dried, three necked flask, with an attached mechanical stirrer, *t*-butylamine (68 mL, 0.3 mol) was added and dissolved in 600 mL of toluene. The mixture was cooled to -30° C, then bromine (16.5 mL, 0.30 mol) was added dropwise over ½ hour. Guaiacol (40 g, 0.3 mol), dissolved in dry CH_2Cl_2 , was added slowly over 1 hour, and the reaction was allowed to warm up to room temperature overnight. The reaction was quenched with Na_2SO_3 (100 mL, 10%) and extracted with ether (3 X 75 mL). The organic layers were combined, dried with $MgSO_4$ and concentrated to yield a brown oil. The oil was purified by two distillations (109-113° C, 3 mm/Hg) to obtain a light yellow oil (39.0 g, 60%);

Note: Data was matched with the previously reported.[49]

^{1}H ($CDCl_3$) 7.12 (dd, J = 7.8, 1.4, 1H), 7.54 (m, 2H), 5.89 (s, 1H), 3.88 (s, 3H).

2,3-dimethoxybromobenzene (148). In a flame-dried round bottom flask, the 2-hydroxy-3-methoxybromobenzene (25..50 g., 0.126 mol) was added to 200 ml of acetone. As the solution stirred, Na_2CO_3 (31.79 g, 0.301 mol) was introduced, followed by the dropwise additon of CH_3I (18.61 mL, 0.301 mol). After allowing to stir for 36 hours, the solid base was filtered off, and the solvent was driven off with the aid of a rotovap. The resulting oil was dissolved in ethyl acetate, and washed with brine (3x 50 ml). The organic layer was then isolated, dried with $MgSO_4$, and concentrated under vacuum. The resulting product crystallized when put in freezer, and yielded 19.29 g (76%).

Note: Data was matched with the previously reported.[162]

H^1 NMR($CDCl_3$) 7.1(dd, J =7.8, 1.7 H_3, 1H), 6.9(t, J =8.3 H_3, 1H), 6.8(dd, J =8.0, 1.5 Hz, IH), 3.8(s,3H), 3.7(s, 3H).

1,2-dimethoxyphenylboronic acid (179). A flame-dried round bottom flask was charged with Mg turnings (2.36 g, 0.098 mol) and a few crystals of iodine. The bromide (17.80 g, 0.083 mol) was then dissolved in 225 ml of dry THF, and added dropwise to the Mg/I_2 mixture. The reaction mixture was allowed to reflux for 3 h, after which triethyl borate (16.71 mL, 0.098 mol) was added dropwise over 5 min. The reaction was quenched with NH_4CL (75 mL, 25%), and washed with ethyl acetate (3 x 50 mL). The organic layers were then combined, washed with brine, and dried with $MgSO_4$. After concentrating the dried organic layers, a light brown solid formed, and yielded 12.9 g (87%).

Note: Data was matched with the previously reported.[163]

^{1}H NMR($CDCl_3$,TMS): 7.36 , (dd, J =5.6, 1.8 H3, 1H), 7.12 (t, J =7.3 Hz, 1H), 7.03 (dd, J =6.3, 1.7 Hz, 1H), 5.97 (bs, 1H), 3.93 (s, 3H), 3.87 (s, 3H), 1.54 (bs, 1H); ^{13}C NMR($CDCl_3$) 55.7(s), 61.37(s), 115.91(s), 124.65(s), 127.43(s).

(2*S*,3*S*)-1-Bromo-5,6-difluoro-2,3-[(isopropylidene)dioxy]cyclohexa-4,6-diene (129). To a stirred solution of **127** (1.13 g, 5.0 mmol) in acetone (100 ml) at r.t. was added neat dimethoxypropane (1.5 ml, 16.8 mmol) dropwise. The solution was stirred for three min and TsOH (45 mg, 0.25 mmol) was added. The reaction was stirred for two h and another 1.5 mL of DMP was added. After one additional hour the reaction was complete (monitored by TLC). The reaction was diluted with CH_2Cl_2 and then quenched with a saturated solution of Na_2CO_3. The layers were separated and the water layer was extracted three times with CH_2Cl_2. The combined organic extracts were dried with $MgSO_4$, and the solvent removed. The resulting oil was purified by column chromatography (hexanes/EtOAc 1:1) to yield **129** (92%)

Colorless oil. $[\alpha]_D^{24}$= +76.9 (*c* 1.0, $CHCl_3$); IR (neat): 2949, 1023, 1197, 1364, 1614; ^{1}H NMR (300 MHz) 5.63 (1H, m), 4.85 (2H, m), 1.43 (3H, s), 1.40 (3H, s); ^{13}C NMR 149.6 (dd, J_1 = 263, 22; C), 103.7 (d, J = 16; C), 102.8 (d, J = 14; CH), 75,8 (s, CH) , 71.0 (d, J = 10; CH), 26.6 (CH), 24.8 (CH) ^{19}F NMR ($CDCl_3$, 300 MHz) -128.95 (m), -124.11(m) MS (EI, 70EV) *m/z* (relative intensity) 268(38, M^+ + $2H(C_9H_{11}{}^{81}BrF_2O_2)$), 267(39, M^+ + $2H(C_9H_{11}{}^{79}BrF_2O_2)$), 253(85), 251(83), 211(97), 209(100), 130(69). HRMS calc. for $C_9H_{11}BrF_2O_2$: 266.9832 (M^++2H), Found: 267.9885.

(2*S*,3*S*)-5,6-Difluoro-2,3-[(isopropylidene)dioxy]cyclohexa-4,6-diene (130). To a stirred solution of **129** (0.42 g, 2.27 mmol) in anh. THF (30 ml) at -78°C was added *tert*-BuLi (1.7 M in pentane, 4.1 mL, 3.4 mmol) dropwise. The solution was stirred for 15

min and MeOH (2.1 ml, 74 mmol) was added dropwise while the temperature was maintained at -78°C. The mixture was stirred for another 15 min and allowed to warm to room temperature. The reaction was diluted with EtOAc, neutralized with NH_4Cl solution, and extracted twice with EtOAc. The organic extract was dried with anh. $MgSO_4$, and the solvent was removed at room temperature under reduced pressure. The resulting oil was purified by column chromatography (hexanes:$CHCl_3$::3:1) to yield **5b** as a yellow oil. (60%) IR (neat): 2989 (w), 2936 (w), 1736 (m), 1652 (w), 1404 (s), 1244 (s), 1212 (s), 1176 (w), 1047 (s), 867 (w), 759 (s); ^{1}H NMR (300 MHz) 5.55 (2H, m), 4.85 (2H, m), 1.45 (3H, s), 1.38 (3H, s); ^{13}C NMR 150.3 (dd, J = 262, 28 Hz, C), 106.2 (s, C), 104.6 (dd, J = 11, 3 Hz, CH), 71.1 (broad s, CH), 27.1 (CH_3), 25.0 (CH_3). ^{19}F NMR (300 MHz, $CDCl_3$) -131,87 (m, J =4.9 Hz) GC-MS(CI) m/z (relative intensity) 188(9, M^+), 130(21), 117(100) 111(76) HRMS calcd. For $C_9H_{10}F_2O_2$: 188.0649 found: 188.0655.

REFERENCES

1. Ley, S. V.; Sternfeld, F.; Taylor, S. *Tetrahedron Lett.* **1987**, *28*, 225.

2. Hudlicky, T. *Chem. Rev.* **1996**, *96*, 3.

3. Hudlicky, T. *ACS Symp. Ser.* 1996, *626*, 180.

4. Carless, H. A. J. *J. Chem. Soc., Chem. Commun.* **1992**, 234.

5. Hudlicky, T.; Entwistle, D. A.; Pitzer, K. K.; Thorpe, A. J. *Chem. Rev.* **1996**, *96*, 1195.

6. Hudlicky, T.; Reed, J. W. *Adv. Asymmetric Synth* **1995**, *1*, 271.

7. Hudlicky, T.; Gonzalez, D.; Gibson, D. T. *Aldrichimica Acta* **1999**, *31*, 35.

8. Gibson, D. T.; Koch, J. R.; Kallio, R. E. *Biochemistry* **1968**, *7*, 2653.

9. Ballard, D. G.; Courtis, A.; Shirley, J. M.; Taylor, S. C. *J. Chem. Soc., Chem. Commun.* **1983**, 954.

10. Allen, C. C. R.; Boyd, D. R.; Dalton, H.; Sharma, N. D.; Haughey, S. A.; McMordie, R. A. S.; McMurray, B. T.; Sheldrake, G. N.; Sproule, K. *J. Chem. Soc., Chem. Commun.* **1995**, 119.

11. Suen, W. C.; Gibson, D. T. *Gene* **1994**, *143*, 67.

12. Zylstra, G. J.; Gibson, D. T. *J. Biol. Chem.* **1989**, *264*, 14940.

13. Gonzalez, D.; Schapiro, V.; Seoane, G.; Hudlicky, T. *Tetrahedron: Asymmetry* **1997**, *8*, 975.

14. Gonzalez, D.; Schapiro, V.; Seoane, G.; Hudlicky, T.; Abboud, K. *J. Org. Chem.* **1997**, *62*, 1194.

15. Gonzalez, D.; Martinot, T.; Hudlicky, T. *Tetrahedron Lett.* **1999**, *40*, 3077.

16. Hartwell, J. L. *Lloydia* **1967**, *30*, 379.

17. Paulsen, H.; Stubbe, M. *Tetrahedron Lett.* **1982**, 3171.

18. Paulsen, H.; Stubbe, M. *Liebigs Ann. Chem.* **1983**, 535.

19. Okamoto, T.; Torii, Y.; Isogai, Y. *Chem. Pharm. Bull.* **1968**, *16*, 1860.

20. Ceriotti, G. *Nature* **1967**, *11*, 595.

21. Rigby, J. H.; Mateo, M. E. *J. Am. Chem. Soc.* **1997**, *119*, 12655.

22. Tian, X.; Maurya, R.; Konigsberger, K.; Hudlicky, T. *Synlett* **1995**, 1125.

23. Pettit, G. R.; Groszek, G.; Backhaus, R. A.; Doubek, D. L.; Barr, R. J.; Meerow, A. W. *J. Nat. Prod.* **1995**, *58*, 756.

24. Pettit, G. R.; Backhaus, R. A.; Boettner, F. E. *J. Nat. Prod.* **1995**, *58*, 37.

25. Pettit, G. R.; Gaddamidi, V.; Herald, D. L.; Singh, S. B.; Cragg, G. M.; Schmidt, J. M.; Boettner, F.; Williams, M.; Sagawa, Y. *J. Nat. Prod.* **1986**, *49*, 995.

26. Tian, X.; Hudlicky, T.; Konigsberger, K. *J. Am. Chem. Soc.* **1995**, *117*, 3643.

27. Hudlicky, T.; Tian, X.; Koenigsberger, K.; Maurya, R.; Rouden, J.; Fan, B. *J. Am. Chem. Soc.* **1996**, *118*, 10752.

28. Piozzi, F.; Fugante, C.; Mondelli, R.; Ceriotti, G. *Tetrahedron* **1968**, *24*, 1119.

29. Evidente, A. *Planta Med.* **1991**, 293.

30. Immirzi, A.; Fuganti, C. *J. Chem. Soc., Chem. Commun.* **1972**, 240.

31. Trimino Ayllon, Z.; Ramos Martinez, I.; Iglesias perez, C.; Spenglers Salabarria, I.; Velez Castro, H. *Rev. Cubana Farm.* **1989**, *23*, 155.

32. Fuganti, F.; Stauton, J.; Battersby, A. R. *J. Chem. Soc., Chem. Commun.* **1971**, 1154.

33. Fuganti, F.; Mazza, M. *J. Chem. Soc., Chem. Commun.* **1971**, 1388.

34. Fuganti, F.; Mazza, M. *J. Chem. Soc., Chem. Commun.* **1972**, 239.

35. Hudlicky, T.; Olivo, H. F. *J. Am. Chem. Soc.* **1992**, *114*, 9694.

36. Chida, N.; Ohtsuka, M.; Ogawa, S. *Tetrahedron Lett.* **1991**, *32*, 4525.

37. Chida, N.; Ohtsuka, M.; Ogawa, S. *J. Org. Chem.* **1993**, *58*, 4441.

38. Martin, S. T. H. *Heterocycles* **1993**, *35*, 85.

39. Pettit, G. R.; Melody, N.; Osullivan, M.; Thompson, M. A.; Herald, D. L.; Coates, B. *J. Chem. Soc., Chem. Commun.* **1994**, 2725.

40. Lopes, R. S. C.; Lopes, C. C.; Heathcock, C. H. *Tetrahedron Lett.* **1992**, *33*, 6775.

41. Mandel, M.; Hudlicky, T. *J. Chem. Soc., Perkin Trans. 1* **1993**, 741.

42. Hudlicky, T.; Olivo, H. F. *Tetrahedron Lett.* **1991**, *32*, 6077.

43. Hudlicky, T.; Luna, H.; Olivo, H. F.; Andersen. C.; Nugent, T.; Price, J. D. *J. Chem. Soc.-Perkin Trans. 1* **1991**, 2907.

44. Hudlicky, T.; Boros, E. E.; Olivo, H. F.; Merola, J. S. *J. Org. Chem.* **1992**, *57*, 1026.

45. Keck, G. E.; Fleming, S.; Nickell, D.; Weider, P. *Synth. Commun.* **1979**, *9*, 281.

46. Olivo, H. F. Ph.D. Dissertation, Virginia Polytechnic Institute and State University, 1992.

47. Hudlicky, T.; Olivo, H. F.; McKibben, B. *J. Am. Chem. Soc.* **1994**, *116*, 5108.

48. Trost, B. M.; Pulley, S. R. *J. Am. Chem. Soc.* **1995**, *117*, 10143.

49. Ishizaki, M.; Ozaki, K.; Kanematsu, A.; Isoda. T.; Hoshino, O. *J. Org. Chem.* **1993**, *58*, 3877.

50. Butora, G.; Hudlicky, T.; Fearnley, S. P.; Gum, A. G.; Stabile, M. R.; Abboud, K. *Tetrahedron Lett* **1996**, *37*, 8155.

51. Butora, G.; Hudlicky, T.; Fearnley, S. P.; Stabile, M. R.; Gum, A. G.; Gonzalez, D. *Synthesis* **1998**, 665.

52. Nakamoto, W. H.; Shiba, T. *Tetrahedron Lett.* **1984**, *25*, 4411.

53. Ireland, C. R.; Durso, A. R.; Newman, R. A.; Hacker, M. P. *J. Org. Chem.* **1982**, *47*, 1807.

54. Biskupiak, J. E.; Ireland, C. R. *J. Org. Chem.* **1983**, *48*, 2302.

55. Kubel, B.; Hofle, G.; Steglich, W. *Angew. Chem. Int. Ed. Eng.* **1975**, *89*, 58.

56. Engel, N.; Kubel, B.; Steglich, W. *Angew. Chem. Int. Ed. Eng.* **1977**, *16*, 394.

57. Bartlett, P. A.; Barstow, J. F. *J. Org. Chem.* **1982**, *47*, 3933.

58. Ireland, R. E.; Mueller, r. H. *J. Am.Chem. Soc.* **1972**, *98*, 2868.

59. Vittorelli, H.-J.; Hansen, P.; Schmid, H. *Helv. Chem. Acta* **1975**, *58*.

60. Kazmaier, U. *Angew. Chem., Int. Edit. Engl.* **1994**, *33*, 998.

61. Kazmaier, U. *Liebigs Ann., Recl.* **1997**, 285.

62. Ireland, R. E.; Wipf, P.; Xiang, J.-N. *J. Org. Chem.* **1991**, *56*, 3572.

63. Kazmaier, U.; Maier, S. *J. Chem. Soc., Chem. Commun.* **1995**, 1991.

64. Kazmaier, U.; Maier, S. *Tetrahedron* **1996**, *52*, 941.

65. Krebs, A.; Kazmaier, U. *Tetrahedron Lett.* **1996**, *37*, 7945.

66. Corey, E. J.; Ley, D. *J. Am. Chem. Soc.* **1991**, *113*, 4026.

67. Kazmaier, U.; Krebs, A. *Angew. Chem.-Int. Edit. Engl.* **1995**, *34*, 2012.

68. Kazmaier, U.; Schneider, C. *Synthesis* **1998**, 1321.

69. Kazmaier, U.; Schneider, C. *Tetrahedron Lett.* **1998**, *39*, 817.

70. Schneider, C.; Kazmaier, U. *Synthesis* **1998**, 1314.

71. Schneider, C.; Kazmaier, U. *Eur. J. Org. Chem.* **1998**, 1155.

72. Kazmaier, U. *J. Org. Chem.* **1994**, *59*, 6667.

73. Kazmaier, U.; Maier, S. *Chem. Commun.* **1998**, 2535.

74. Hudlicky, T.; Butora, G.; Fearnley, S. P.; Gum, A. G.; Stabile, M. R. In *Studies in Natural Products Chemistry*; Atta-ur-Rahman, Ed.; Elsevier: Amsterdam, 1996, p 43.

75. Gates, M.; Tschudi, G. *J. Am. Chem. Soc.* **1952**, *74*, 1109.

76. Ginsburg, D.; Elad, D. *J. Am. Chem. Soc.* **1954**, *76*, 312.

77. Moos, W. H.; Gless, R. D.; Rapoport, H. *J. Org. Chem.* **1982**, *48*, 227.

78. Chandler, M.; Parsons, P. J. *J. Chem. Soc., Chem. Commun.* **1984**, 322.

79. Parsons, P. J.; Penkett, C. S.; Shell, A. J. *Chem. Rev.* **1996**, *96*, 195.

80. Mulzer, J.; Bats, J. W.; List, B.; Opatz, T.; Trauner, D. *Synlett* **1997**, 441.

81. Evans, D. A.; Mitch, C. H. *Tetrahedron Lett.* **1982**, *23*, 285.

82. Weller, D. D.; Rapoport, H. *J. Am. Chem. Soc.* **1976**, *98*, 6650.

83. Mulzer, J.; Durner, G.; Trauner, D. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2830.

84. Trauner, D.; Bats, J. W.; Werner, A.; Mulzer, J. *J. Org. Chem.* **1998**, *63*, 5908.

85. Ghosh, R.; Robinson, R. *J. Chem. Soc.* **1944**, 506.

86. Gibson, D. T.; Koch, J. R.; Kallio, R. E. *Biochemistry* **1968**, 3795.

87. Ribbons, D. W.; Cass, A. E. G.; Rossiter, J. T.; Taylor, S. J. C.; Woodland, M. P.; Widdowson, D. A.; Williams, S. R.; Baker, P. B.; Martin, R. E. *J. Fluorine Chem.* **1987**, *37*, 299.

88. Martin, R. E.; Baker, P. B.; Ribbons, D. W. *Biocatalysis* **1987**, *1*, 37.

89. Rossiter, J. T.; Williams, S. R.; Cass, A. E. G.; Ribbons, D. W. *Tetrahedron Lett.* **1987**, *28*, 5173.

90. Davey, J. F.; Gibson, D. T. *J. Bacteriol.* **1974**, *119*, 923.

91. Gibson, D. T.; Mahadevan, V.; Davey, J. F. *J. Bacteriol.* **1974**, *119*, 930.

92. Ziffer, H.; Kabuto, K. P.; Gibson, D. T.; Kobal, V. M.; Jerina, D. M. *Tetrahedron Lett.* **1977**, *33*, 2491.

93. DeFrank, J. J.; Ribbons, D. W. *J. Bacteriol.* **1977**, *29*, 1356.

94. Knackmuss, H. J. *Chem.-Ztg.* **1975**, *99*, 213.

95. *Fluorinated Carbohydrates: Chemical and Biochemical Aspects*; ACS: Washington, DC, 1988.

96. Cass, A. E. G.; Ribbons, D. W.; Rossiter, J. T.; Williams, S. R. *FEBS Lett.* **1987**, *220*, 353.

97. Yan, F. Y.; Nguyen, B. V.; York, C.; Hudlicky, T. *Tetrahedron* **1997**, *53*, 11541.

98. Nguyen, B. V.; York, C.; Hudlicky, T. *Tetrahedron* **1997**, *53*, 8807.

99. Allen, C. C. R.; Boyd, D. R.; Dalton, H.; Sharma, N. D.; Brannigan, I.; Kerley, N. A.; Sheldrake, G. N.; Taylor, S. C. *J. Chem. Soc., Chem. Commun.* **1995**, 117.

100. Akgun, H.; Hudlicky, T. *Tetrahedron Lett.* **1999**, *40*, 3081.

101. Resnick, S. M.; Torok, D. S.; Gibson, D. T. *J. Org. Chem.* **1995**, *60*, 3546.

102. Hudlicky, T.; Gonzalez, D.; Stabile, M.; Endoma, M. A.; Deluca, M.; Parker, D.; Gibson, D. T.; Resnick, S. M.; Whited, G. M. *J. Fluor. Chem.* **1998**, *89*, 23.

103. Gibson, D. T.; Roberts, R. L.; Wells, M. C.; Kobal, V. M. *Biochem. Biophys. Res. Commun.* **1973**, *50*, 211.

104. Catelani, D.; Sorlini, C.; Treccani, V. *Experientia* **1971**, *27*, 1173.

105. Gibson, D. T.; Subramanian, V.; Ensley, B. D.; Reineke, W.; Safe, S. H. In *Microbial Degradation of Organic Compounds*; Gibson, D. T., Ed.; Marcel Dekker: New York, 1984.

106. Furukawa, K.; Tomizuka, N.; Kamibayashi, A. *Appl. Environ. Microbiol.* **1979**, *38*, 301.

107. Kimura, K.; Kato, H.; Nishi, A.; Furukawa, K. *Biosci. Biotech. Biochem.* **1996**, *60*, 220.

108. Ley, S. V.; Redgrave, A. J.; Taylor, S. C.; Ahmed, S.; Ribbons, D. W. *Synlett* **1991**, 741.

109. Pearson, D. E.; Wysong, R. D.; Breder, C. V. *J. Org. Chem.* **1967**, *32*, 2358.

110. Stille, J. K. *Pure Appl. Chem.* **1985**, *57*, 1771.

111. Stille, J. K. *Agew. Chem. Int. Ed. Engl.* **1986**, *25*, 508.

112. Miyaura, N.; Yanagi, T.; Suzuki, A. *Synth. Commun.* **1981**, *11*, 513.

113. Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457.

114. O'Keefe, D. F.; Dannock, M. C.; Marcuccio, S. B. *Tetrahedron Lett.* **1992**, *33*, 6679.

115. Dallacker, F.; Sanders, J. *Chem. -Ztg.* **1984**, *108*, 186.

116. Shirasaka, T.; Takuma, Y.; Imaki, N. *Synth. Commun.* **1990**, *20*, 1223.

117. Hudlicky, T.; Boros, E. E. *Tetrahedron: Asymmetry* **1992**, *3*, 217.

118. Berson *J. Am. Chem. Soc.* **1969**, *91*, 5569.

119. McKean, D. R.; Parrinello, G.; Renaldo, A. F.; Stille, J. K. *J. Org. Chem.* **1987**, *52*, 422.

120. Heck, R. F. *Palladium Reagents in Organic Synthesis*; Academic Press: New York, 1985.

121. Boyd, D. R.; Sharma, N. D.; Barr, S. A.; Dalton, H.; Chima, J.; Whited, G.; Seemayer, R. *J. Am. Chem. Soc.* **1994**, *116*, 1147.

122. Boyd, D. R.; Dorrity, M. R. J.; Hand, M. V.; Malone, J. F.; Sharma, N. D.; Dalton, H.; Gray, D. J.; Sheldrake, G. N. *J. Am. Chem. Soc.* **1991**, *113*, 666.

123. Boyd, D. R.; Sharma, N. D.; Byrne, B.; Hand, M. V.; Malone, J. F.; Sheldrake, G. N.; Blacker, J.; Dalton, H. *J. Chem. Soc.-Perkin Trans. 1* **1998**, 1935.

124. Hudlicky, T.; Luna, H.; Barbieri, G.; Kwart, L. D. *J. Am. Chem. Soc.* **1988**, *110*, 4735.

125. Gibson. D. T.; Hensley, M.; Yoshioka, H.; Mabry, T. J. *Biochemistry* **1970**, *9*, 1626.

126. Hudlicky, T.; Luna, H.; Price, J. D.; Rulin, F. *Tetrahedron Lett.* **1989**, *30*, 4053.

127. Fu, J.-m.; Snieckus, V. *Tetrahedron Lett.* **1990**, *31*, 1665.

128. Bei, X. H.; Crevier, T.; Guram, A. S.; Jandeleit, B.; Powers, T. S.; Turner, H. W.; Uno, T.; Weinberg, W. H. *Tetrahedron Lett.* **1999**, *40*, 3855.

129. Lohse, O.; Thevenin, P.; Waldvogel, E. *Synlett* **1999**, 45.

130. Littke, A. F.; Fu, G. C. *Angew. Chem.-Int. Edit.* **1998**, *37*, 3387.

131. Shen, W. *Tetrahedron Lett.* **1997**, *38*, 5575.

132. Johnson, C. R.; Johns, B. A. *J. Org. Chem.* **1997**, *62*, 6046.

133. Anderson, J. C.; Namli, H.; Roberts, C. A. *Tetrahedron* **1997**, *53*, 15123.

134. Old, D. W.; Wolfe, J. P.; Buchwald, S. L. *J. Am. Chem. Soc.* **1998**, *120*, 9722.

135. Bumagin, N. A.; Bykov, V. V. *Tetrahedron* **1997**, *53*, 14437.

136. Wetter, H.; Oertle, K. *Tetrahedron Lett.* **1985**, *26*, 5515.

137. Butora, G.; Gum, A. G.; Hudlicky, T.; Abboud, K. A. *Synthesis* **1998**, 275.

138. Hassner, A.; Alexanian, V. *Tetrahedron Lett.* **1978**, 4475.

139. Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

140. Kazmaier, U. *Tetrahedron* **1994**, *50*, 12895.

141. Ireland, R. E.; Maienfisch, P. *J. Org. Chem.* **1988**, *53*, 640.

142. Wipf, P. In *Comprehensive Organic Synthesis*; Trost, B. M., Ed.; Pergamon Press: Oxford, 1991; Vol. 5, p 827.

143. Colombo, L.; Casiraghi, G.; Pittalis, A.; Rassu, G. *J. Org. Chem.* **1991**, *56*, 3897.

144. Ginsburg, D.; Pappo, R. *J. Chem. Soc.* **1953**, 1524.

145. Hudlicky, T.; Price, J. D.; Rulin, F.; Tsunoda, T. *J. Am. Chem. Soc.* **1990**, *112*, 9439.

146. Hudlicky, T.; Fan, R. L.; Tsunoda, T.; Luna, H.; Andersen, C.; Price, J. D. *Isr. J. Chem.* **1991**, *31*, 229.

147. Hudlicky, T.; Fan, R.; Luna, H.; Olivo, H.; Price, J. *Pure Appl. Chem.* **1992**, *64*, 1109.

148. Hudlicky, T.; Boros, C. H. *Tetrahedron Lett.* **1993**, *34*, 2557.

149. Keck, G. E.; McHardy, S. F.; Wager, T. T. *Tetrahedron Lett.* **1995**, *36*, 7419.

150. Cicchi, S.; Goti, A.; Brandi, A.; Guarna, A.; Desarlo, F. *Tetrahedron Lett.* **1990**, *31*, 3351.

151. Ritter, A. R.; Miller, M. J. *J. Org. Chem.* **1994**, *59*, 4602.

152. Geneste, F.; Racelma, N.; Moradpour, A. *Synth. Commun.* **1997**, *27*, 957.

153. Olivo, H. F.; Hemenway, M. S.; Hartwig, A. C.; Chan, R. *Synlett* **1998**, 247.

154. Narashimhan, S.; Balakumar, R. *Aldrichimica Acta* **1998**, *31*, 19.

155. Paquette, L. A. *Encyclopedia of Reagents for Organic Synthesis*; J. Wiley: New York, 1995; Vol. 8.

156. Hoveyda, A. H.; Evans, D. A.; Fu, G. C. *Chem. Rev.* **1993**, *93*, 1307.

157. Nakata, T.; Tanaka, T.; Oishi, T. *Tetrahedron Lett.* **1983**, *24*, 2653.

158. Banwell, M. G.; Bissett, B. D.; Busato, S.; Cowden, C. J.; Hockless, D. C. R.; Holman, J. W.; Read, R. W.; Wu, A. W. *J. Chem. Soc., Chem. Commun.* **1995**, 2551.

159. Banwell, M. G.; Cowden, C. J.; Gable, R. W. *J. Chem. Soc.-Perkin Trans. 1* **1994**, 3515.

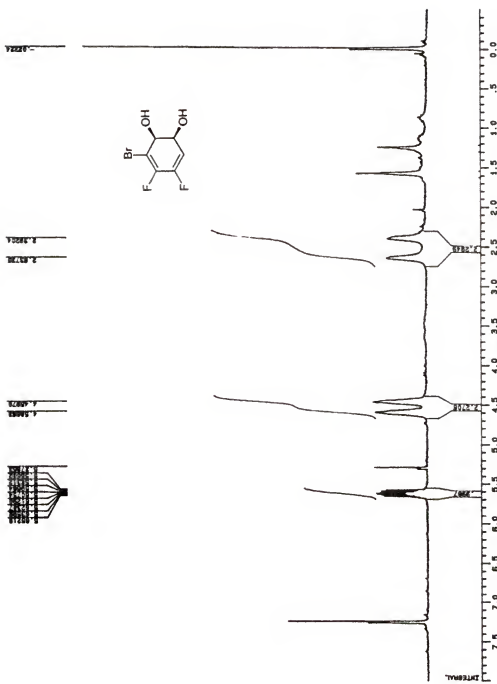
160. Hudlicky, T.; Stabile, M. S.; Whited, G.; Gibson, D. T. *Organic Synthesis* **1999**, *76*, 77.

161. Hudlicky, T.; Boros, C. H.; Boros, E. E. *Synthesis* **1992**, 174.

162. Stevens, R.; Bisacchi, G. *J. Org. Chem.* **1982**, *47*, 2396.

163. Dang, Q. *Bull. Soc. Chim. Fr.* **1973**, 767.

APPENDIX
SELECTED SPECTRA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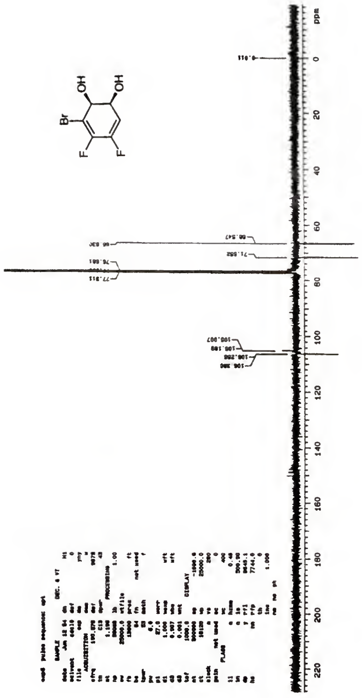

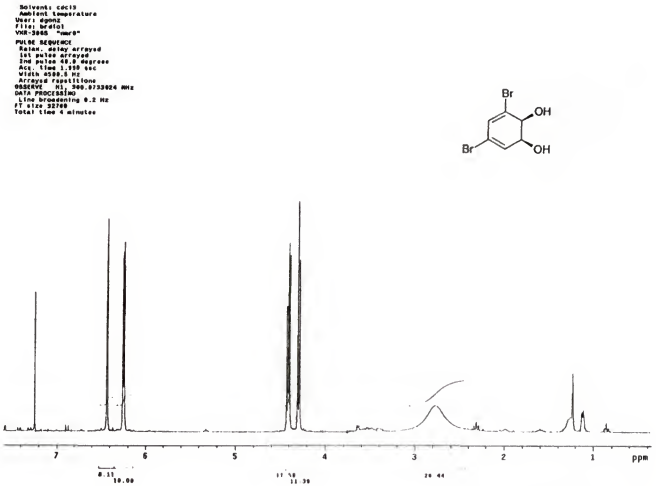
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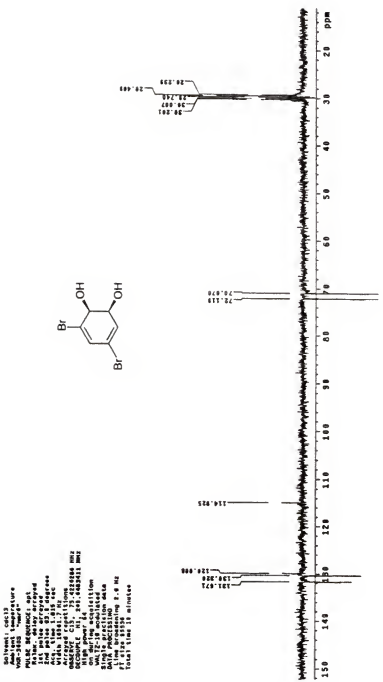
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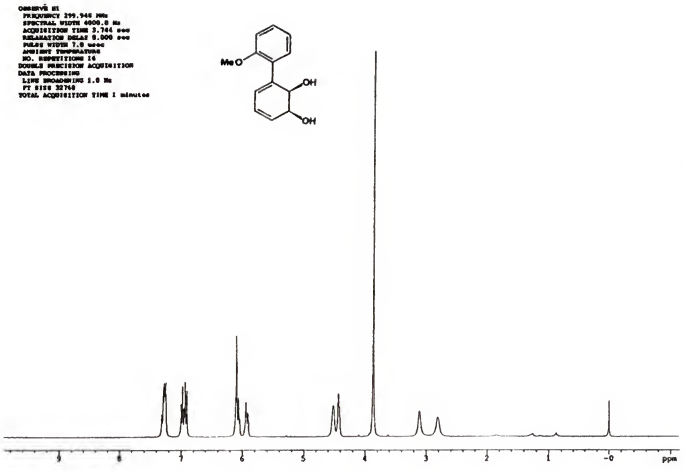
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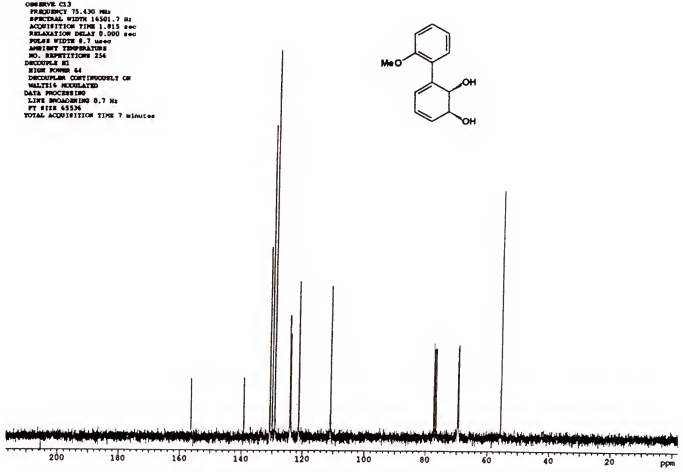
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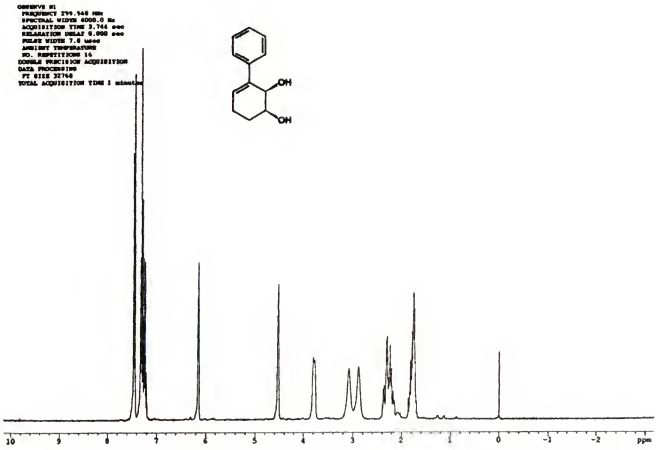
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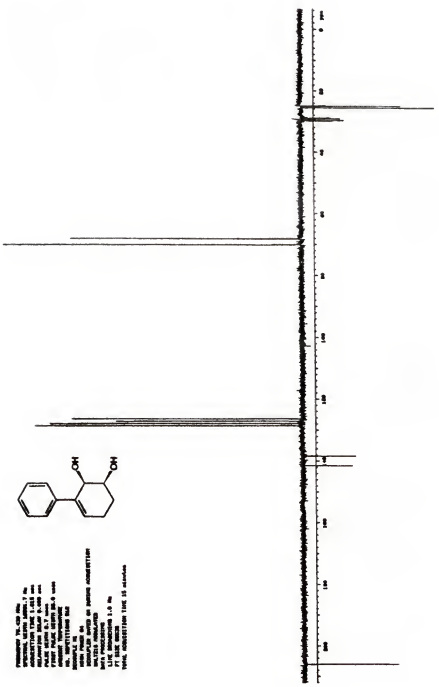

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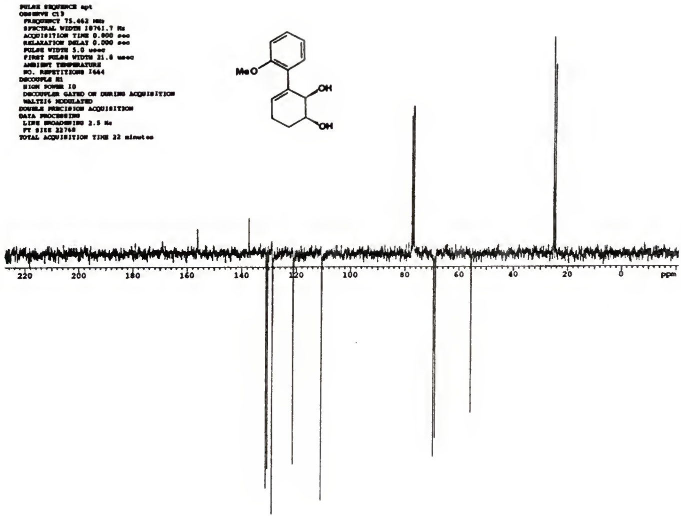
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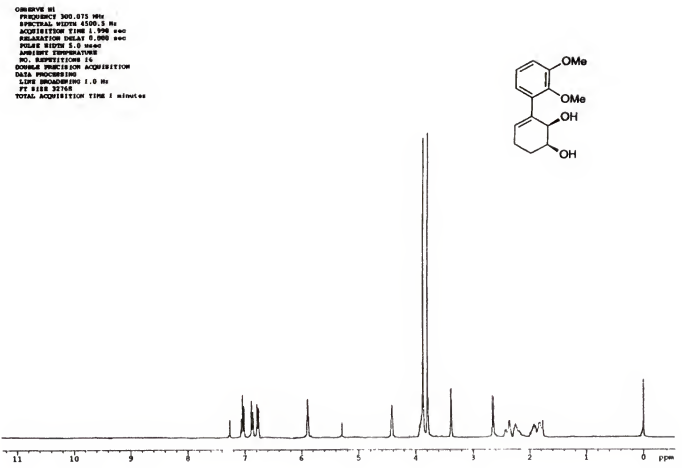
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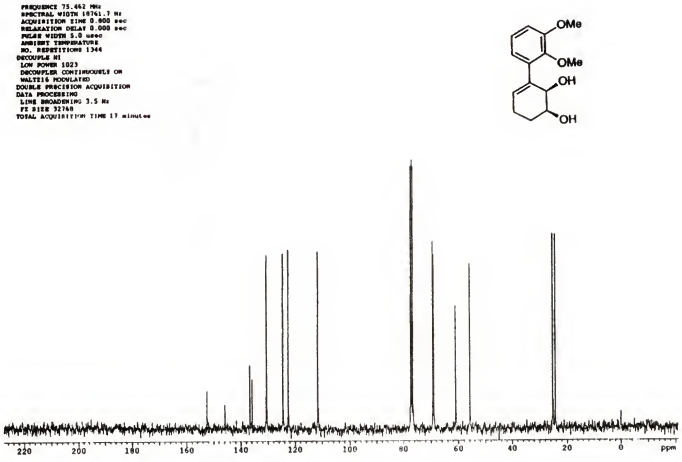
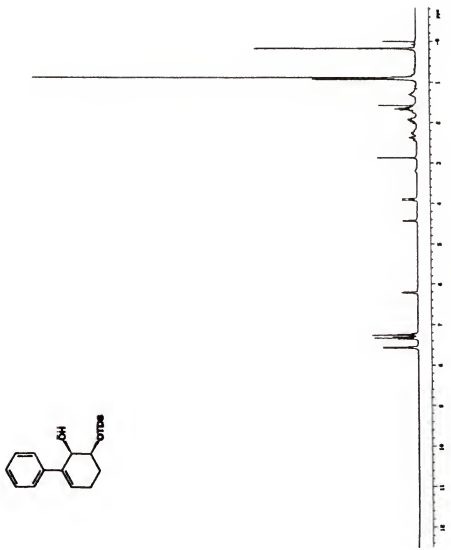
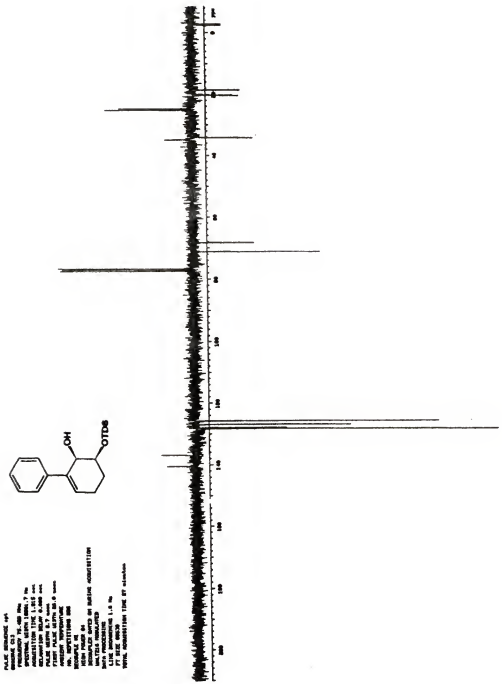
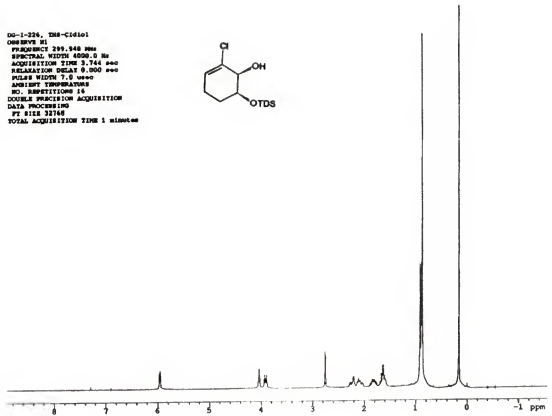
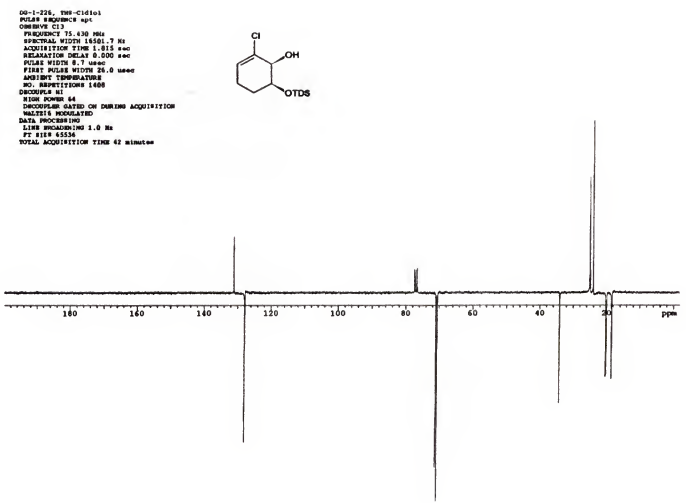
11
10
9
8
7
6
5
4
3
2
1
0
ppm

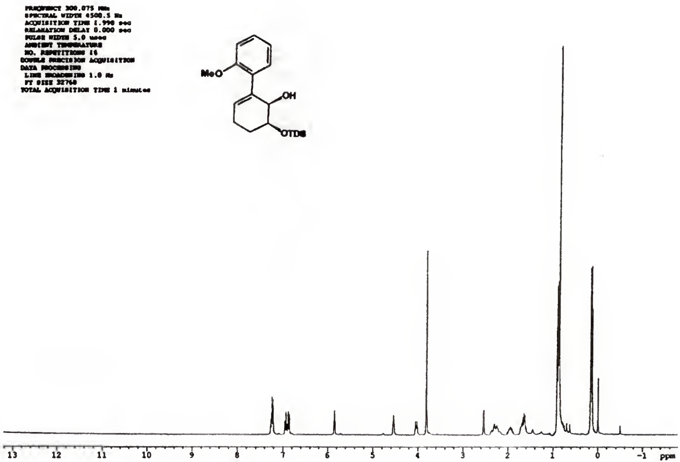

FREQUENCY 75.462 MHz
SPECTRAL WIDTH 18761.7 Hz
ACQUISITION TIME 0.800 sec
RELAXATION DELAY 0.000 sec
PULSE WIDTH 5.0 usec
AMBIENT TEMPERATURE
NO. REPETITIONS 1344
DECOUPLE H1
LOW POWER 1023
DECOUPLER CONTINUOUSLY ON
WALTZ16 MODULATED
DOUBLE PRECISION ACQUISITION
DATA PROCESSING
LINE BROADENING 3.5 Hz
FT SIZE 32768
TOTAL ACQUISITION TIME 17 minutes
OMe
OMe
OH
OH
220
200
180
160
140
120
100
80
60
40
20
0
ppm

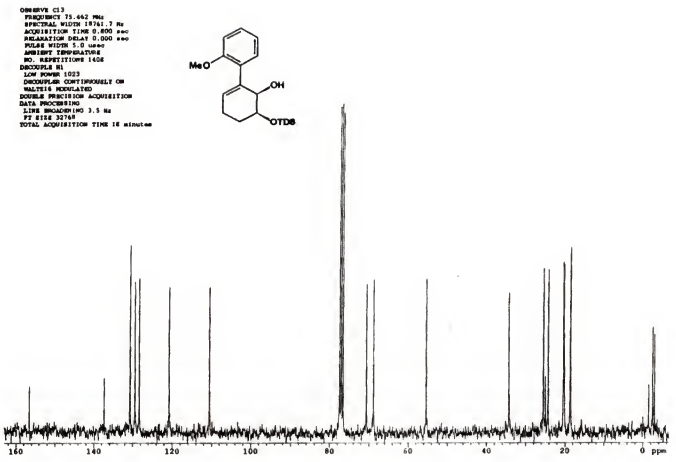

OH
OTBS


OH
OTBS

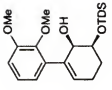

DG-1-326, TMS-Cidiol
OBSERVE H1
FREQUENCY 299.946 MHz
SPECTRAL WIDTH 4098.0 Hz
ACQUISITION TIME 3.744 sec
RELAXATION DELAY 0.000 sec
PULSE WIDTH 7.0 usec
AMBIENT TEMPERATURE
NO. REPETITIONS 16
DOUBLE PRECISION ACQUISITION
DATA PROCESSING
FT SIZE 32768
TOTAL ACQUISITION TIME 1 minutes
Cl
OH
OTDS
8
7
6
5
4
3
2
1
0
-1 ppm

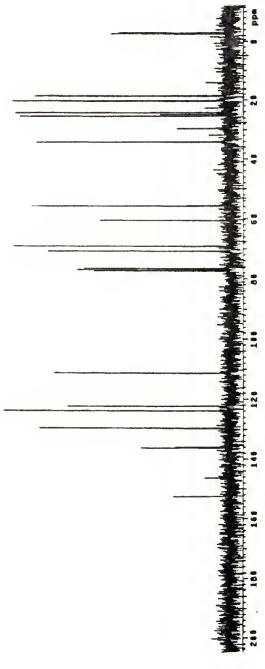

DG-1-226, THS-Cidiol
PULSE SEQUENCE sgt
OBSERVE C13
FREQUENCY 75.430 MHz
SPECTRAL WIDTH 16501.7 Hz
ACQUISITION TIME 1.815 sec
RELAXATION DELAY 0.000 sec
PULSE WIDTH 8.7 usec
FIRST PULSE WIDTH 26.0 usec
AMBIENT TEMPERATURE
NO. REPETITIONS# 1408
DECOUPLE H1
HIGH POWER 64
DECOUPLER GATED ON DURING ACQUISITION
WALTZ16 MODULATED
DATA PROCESSING
LINE BROADENING 1.0 Hz
FT SIZE 65536
TOTAL ACQUISITION TIME 42 minutes
Cl
OH
OTDS
180
160
140
120
100
80
60
40
20
ppm


FREQUENCY 300.075 MHz
SPECTRAL WIDTH 4500.5 Hz
ACQUISITION TIME 1.990 sec
RELAXATION DELAY 0.000 sec
PULSE WIDTH 5.0 usec
AMBIENT TEMPERATURE
NO. REPETITIONS 16
DOUBLE PRECISION ACQUISITION
DATA PROCESSING
LINE BROADENING 1.0 Hz
FT SIZE 32768
TOTAL ACQUISITION TIME 1 minutes
MeO
OH
OTDS
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
ppm


OBSERVE C13
FREQUENCY 75.462 MHz
SPECTRAL WIDTH 19761.7 Hz
ACQUISITION TIME 0.800 sec
RELAXATION DELAY 0.000 sec
PULSE WIDTH 5.0 usec
AMBIENT TEMPERATURE
NO. REPETITIONS 1406
DECOUPLE H1
LOW POWER 1023
DECOUPLER CONTINUOUSLY ON
WALTZ16 MODULATED
DOUBLE PRECISION ACQUISITION
DATA PROCESSING
LINE BROADENING 3.5 Hz
FT SIZE 32768
TOTAL ACQUISITION TIME 16 minutes
MeO
OH
OTDS
160
140
120
100
80
60
40
20
0 ppm

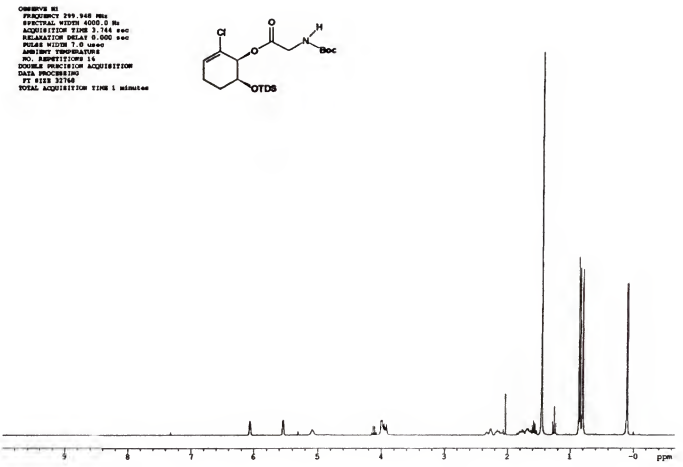
OMe
OMe
OH
OTDS
ppm

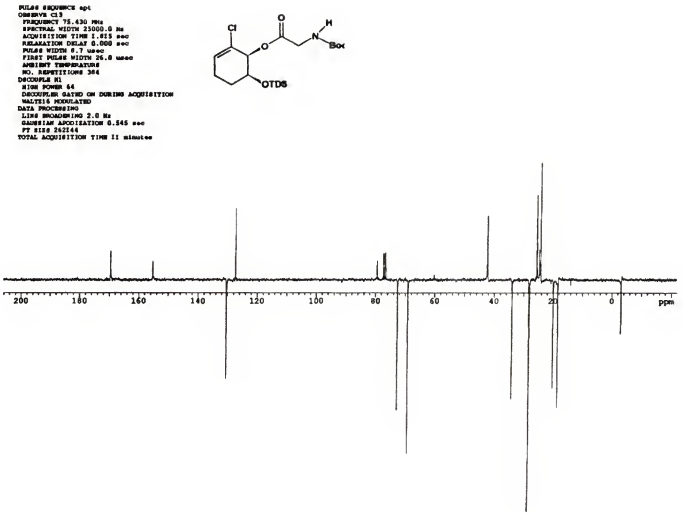

OMe
OMe
OH
OTDS




OTDS
Boc
ppm

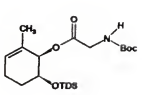
OTDS
Boc
ppm

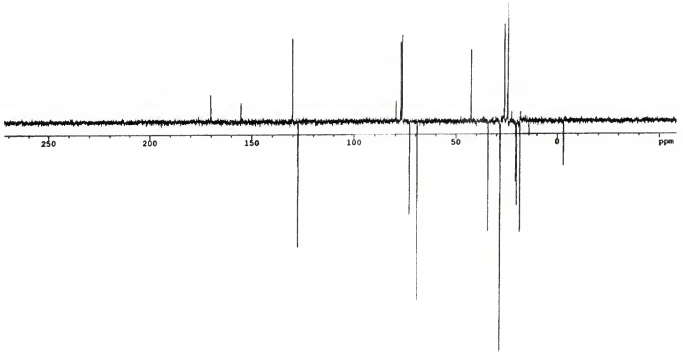

OBSERVE H1
FREQUENCY 299.948 MHz
SPECTRAL WIDTH 4000.0 Hz
ACQUISITION TIME 3.744 sec
RELAXATION DELAY 0.000 sec
PULSE WIDTH 7.0 usec
AMBIENT TEMPERATURE
NO. REPETITIONS 16
DOUBLE PRECISION ACQUISITION
DATA PROCESSING
FT SIZE 32768
TOTAL ACQUISITION TIME 1 minutes
Cl
O
O
N
H
Boc
OTDS
9
8
7
6
5
4
3
2
1
-0
ppm


PULSE SEQUENCE opt
OBSERVE C13
FREQUENCY 75.430 MHz
SPECTRAL WIDTH 23000.0 Hz
ACQUISITION TIME 1.815 sec
RELAXATION DELAY 0.000 sec
PULSE WIDTH 6.7 usec
FIRST PULSE WIDTH 26.0 usec
AMBIENT TEMPERATURE
NO. REPETITIONS 384
DECOUPLE H1
NIMS POWER 64
DECOUPLER GATED ON DURING ACQUISITION
WALTZ16 MODULATED
DATA PROCESSING
LINE BROADENING 2.0 Hz
GAUSSIAN APODIZATION 0.545 sec
FT SIZE 262144
TOTAL ACQUISITION TIME 11 minutes
Cl
O
O
N
H
Boc
OTDS
200
180
160
140
120
100
80
60
40
20
0
ppm

OBSERVE H1
PROTON 399.948 MHz
SPECTRAL WIDTH 4139.9 Hz
ACQUISITION TIME 3.9541 sec
RELAXATION DELAY 0.000 sec
PULSE WIDTH 7.4 usec
AMBIENT TEMPERATURE
NUMBER OF SCANS 32
DOUBLE PRECISION ACQUISITION
DATA BLOCKED IN MEMORY
FT SIZE 32768
TOTAL ACQUISITION TIME 2 minutes
O
N
H
Boc
CH3
OTDS
10
9
8
7
6
5
4
3
2
1
0
-1
-2
ppm

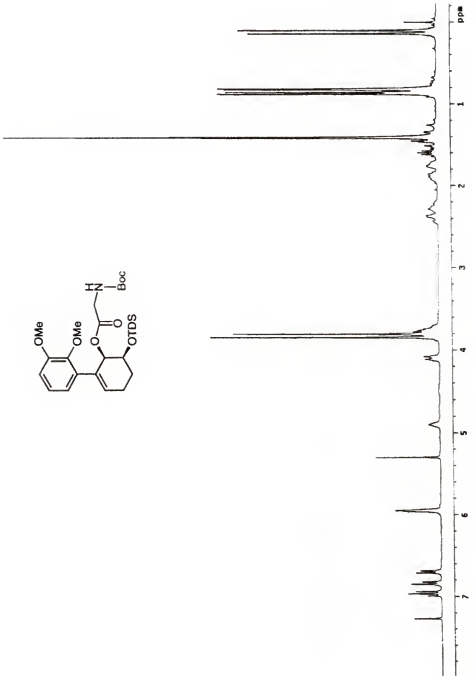
PULSE SEQUENCE apt
OBSERVE C13
FREQUENCY 75.430 MHz
SPECTRAL WIDTH 25000.0 Hz
ACQUISITION TIME 1.815 sec
RELAXATION DELAY 0.000 sec
PULSE WIDTH 8.7 usec
FIRST PULSE WIDTH 26.0 usec
AMBIENT TEMPERATURE
NO. REPETITIONS 928
DECOUPLE H1
HIGH POWER 64
DECOUPLER GATED ON DURING ACQUISITION
MULTIPLE MODULATED
DATA PROCESSING
LINE BROADENING 2.0 Hz
GAUSSIAN APODIZATION 0.545 sec
FT SIZE 262144
TOTAL ACQUISITION TIME 28 minutes

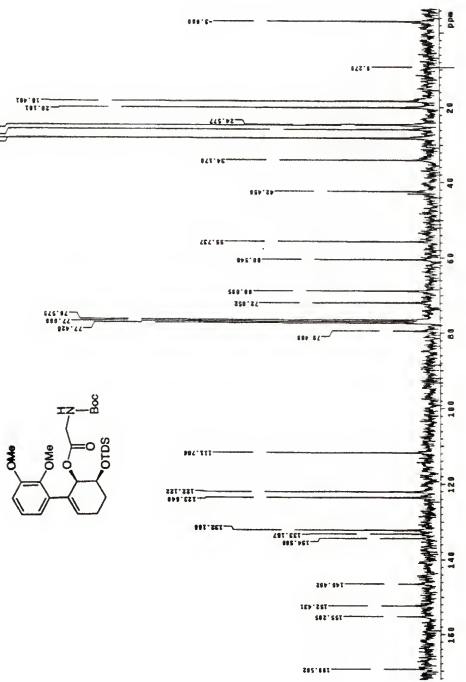


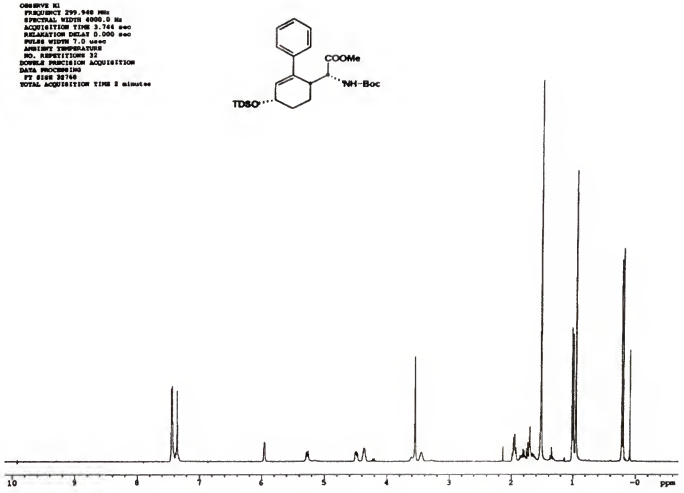


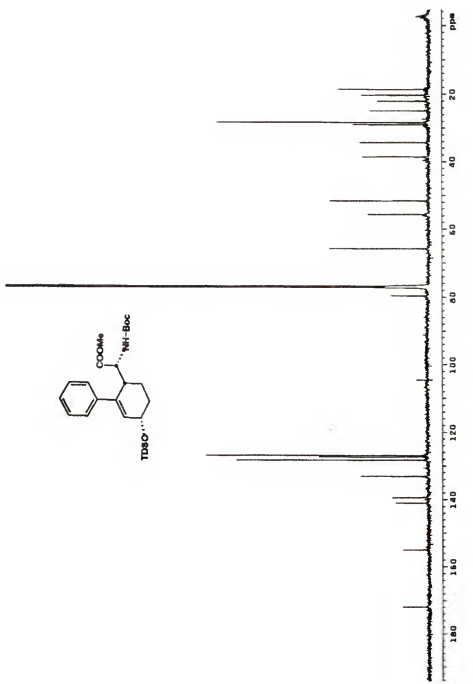
CHROMOX H1
PROTON 300.075 MHz
SPECTRAL WIDTH 4500.5 Hz
FLIP ANGLE 30.0 degrees
RELAXATION DELAY 0.000 sec
ACQUISITION TIME 1.818 sec
ADJUST TEMPERATURE
NUMBER OF REPETITIONS 4
DOUBLE PRECISION ACQUISITION
DATA PROCESSING
LINE BROADENING 1.0 Hz
FT SIZE 65536
TOTAL ACQUISITION TIME 1 minutes
MeO
OTBS
Boc
ppm

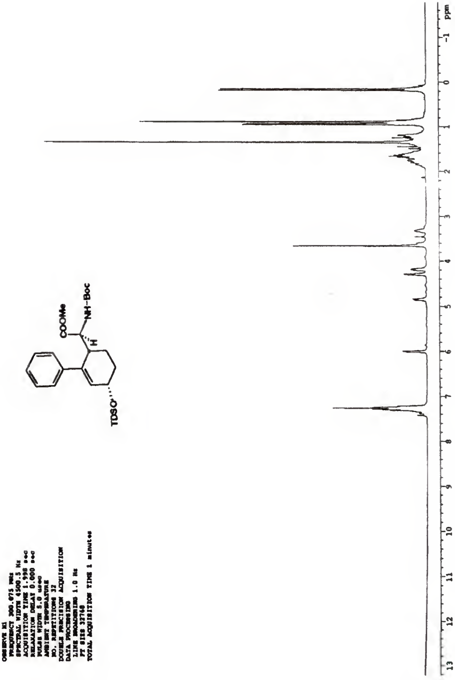
MeO
OTDS
Boc
ppm


OMe
OMe
O
O
N
H
Boc
OTDS
ppm
1
2
3
4
5
6
7

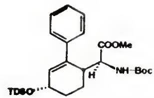

OMe
OMe
O
HN
Boc
O
OTDS

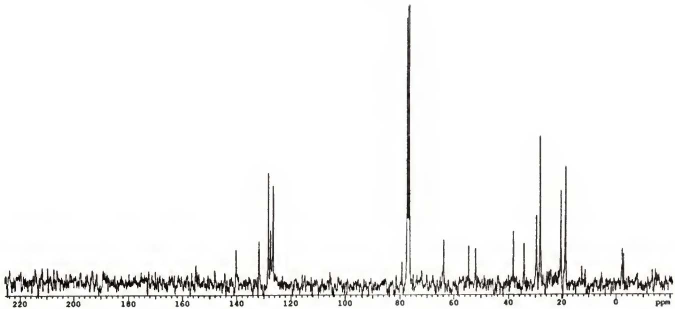

OBSERVE H1
FREQUENCY 299.940 MHz
SPECTRAL WIDTH 4000.0 Hz
ACQUISITION TIME 3.744 sec
RELAXATION DELAY 0.000 sec
PULSE WIDTH 7.0 usec
AMBIENT TEMPERATURE
NO. REPETITIONS 32
DOUBLE PRECISION ACQUISITION
DATA PROCESSING
FT SIZE 32768
TOTAL ACQUISITION TIME 2 minutes
COOMe
NH-Boc
TDSO
10
9
8
7
6
5
4
3
2
1
0
ppm

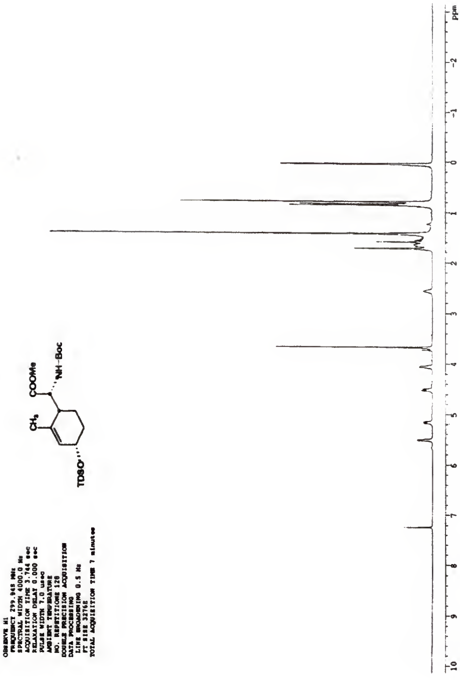

COOMe
NH-Boc
TDSO
ppm
20
40
60
80
100
120
140
160
180


OBSERVE H1
FREQUENCY 300.075 MHz
SPECTRAL WIDTH 4500.5 Hz
ACQUISITION TIME 1.998 sec
RELAXATION DELAY 0.000 sec
PULSE WIDTH 5.0 usec
AMBIENT TEMPERATURE
NO. REPETITIONS 32
DOUBLE PRECISION ACQUISITION
DATA PROCESSING
LINE BROADENING 1.0 Hz
FT SIZE 32768
TOTAL ACQUISITION TIME 1 minutes
COOMe
NH-Boc
H
TDSO
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
ppm

OBSERVE C13
FREQUENCY 75.462 MHz
SPECTRAL WIDTH 18761.7 Hz
ACQUISITION TIME 0.800 sec
RELAXATION DELAY 0.000 sec
PULSE WIDTH 5.0 usec
AMBIENT TEMPERATURE
NO. REPETITIONS 960
DECOUPLE H1
LOW POWER 1023
DECOUPLER CONTINUOUSLY ON
WALTZ16 MODULATED
DOUBLE PRECISION ACQUISITION
DATA PROCESSING
LINE BROADENING 9.9 Hz
FT SIZE 32768
TOTAL ACQUISITION TIME 12 minutes






OBSERVE H1
FREQUENCY 299.948 MHz
SPECTRAL WIDTH 4000.0 Hz
ACQUISITION TIME 3.744 sec
RELAXATION DELAY 0.000 sec
PULSE WIDTH 7.0 usec
AMBIENT TEMPERATURE
NO. REPETITIONS 128
DOUBLE PRECISION ACQUISITION
DATA PROCESSING
LINE BROADENING 0.5 Hz
FT SIZE 32768
TOTAL ACQUISITION TIME 7 minutes
CH3
COOMe
NH-Boc
TDSO
10
9
8
7
6
5
4
3
2
1
0
-1
-2
ppm

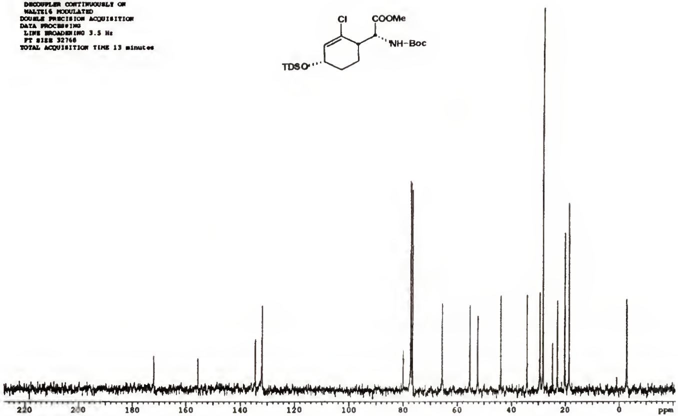
CH3
COOMe
NH-Boc
TDSO
ppm
0
20
40
60
80
100
120
140
160
180
200
220

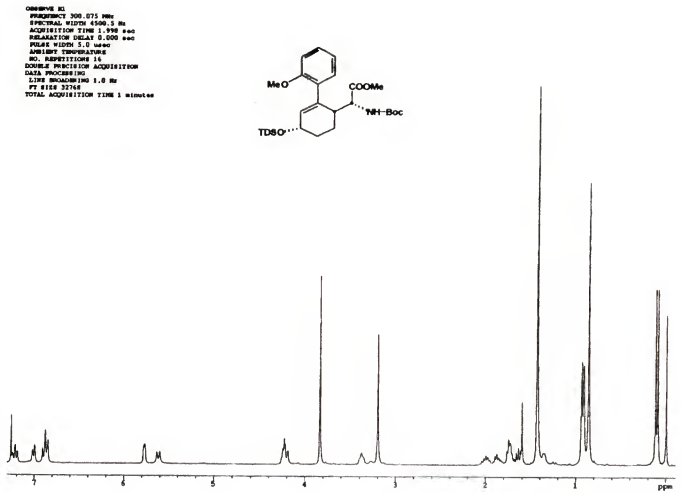
CH3
COOMe
NH-Boc
H
TDSO
ppm

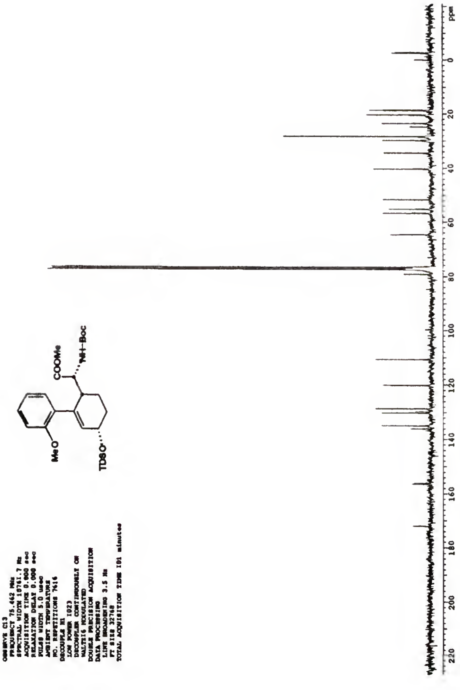
OMBBOX (1)
OBSERVE C13
SPECTRAL WIDTH 18761.3 Hz
PULSE WIDTH 4.5 usec
RELAXATION DELAY 2.000 sec
ACQUISITION TIME 1.3 sec
AMBIENT TEMPERATURE
NUMBER OF SCANS 1066
DECOUPLE H1
FREQUENCY 75.433 MHz
DECOUPLER CONTINUOUS ON
POWER 10.0 dB
DOUBLE PRECISION ACQUISITION
DATA POINTS 32768
LINE BROADENING 3.5 Hz
FT SIZE 131072
TOTAL ACQUISITION TIME 14 minutes
CH3
COOMe
NH-Boc
TDSiO
250
240
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
PPM

OMEGA2 H1
399.975 MHz
SPECTRAL WIDTH 6399.8 Hz
FREQUENCY RANGE 16.000 ppm
ACQUISITION TIME 2.5587 sec
RELAXATION DELAY 0.000 sec
PULSE WIDTH 45.0 degrees
AMBIENT TEMPERATURE
NUMBER OF SCANS 8
DUMMY PRECISION ACQUISITION
DATA PROCESSING
LINE BROADENING 1.0 Hz
FT SIZE 32768
TOTAL ACQUISITION TIME 1 minutes
Cl
COOMe
NH-Boc
TDSO
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
ppm

OBSERVE C13
FREQUENCY 75.462 MHz
SPECTRAL WIDTH 18761.7 Hz
ACQUISITION TIME 0.800 sec
RELAXATION DELAY 0.000 sec
PULSE WIDTH 5.0 usec
AMBIENT TEMPERATURE
NO. REPETITIONS 1024
DECOUPLE H1
LOW POWER 1023
DECOUPLER CONTINUOUSLY ON
WALTZ16 MODULATED
DOUBLE PRECISION ACQUISITION
DATA PROCESSING
LINE BROADENING 3.5 Hz
FT SIZE 32768
TOTAL ACQUISITION TIME 13 minutes

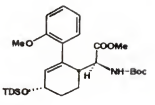


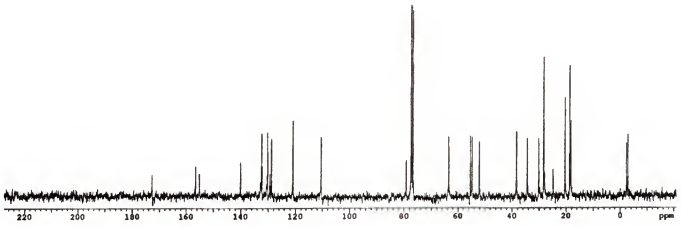

OBSERVE H1
FREQUENCY 300.075 MHz
SPECTRAL WIDTH 4500.1 Hz
ACQUISITION TIME 1.998 sec
RELAXATION DELAY 0.000 sec
PULSE WIDTH 5.0 usec
AMBIENT TEMPERATURE
NO. REPETITIONS 16
DOUBLE PRECISION ACQUISITION
DATA PROCESSING
LINE BROADENING 1.0 Hz
FT SIZE 32768
TOTAL ACQUISITION TIME 1 minutes
MeO
COOMe
NH-Boc
TDSO
7
6
5
4
3
2
1
ppm

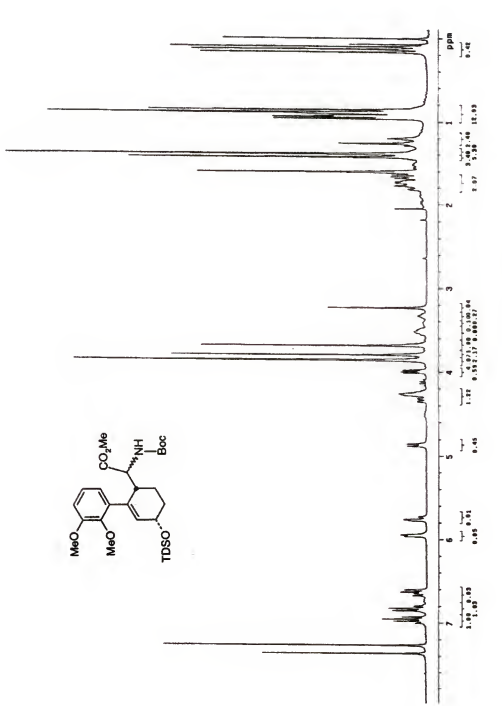

OBSERVE C13
FREQUENCY 75.462 MHz
SPECTRAL WIDTH 18761.7 Hz
ACQUISITION TIME 0.800 sec
RELAXATION DELAY 0.000 sec
PULSE WIDTH 5.0 usec
AMBIENT TEMPERATURE
NO. REPETITIONS 7616
DECOUPLE H1
LOW POWER 1023
DECOUPLER CONTINUOUSLY ON
WALTZ16 MODULATED
DOUBLE PRECISION ACQUISITION
DATA PROCESSING
LINE BROADENING 3.5 Hz
FT SIZE 32768
TOTAL ACQUISITION TIME 101 minutes
MeO
COOMe
NH-Boc
TDSO
ppm

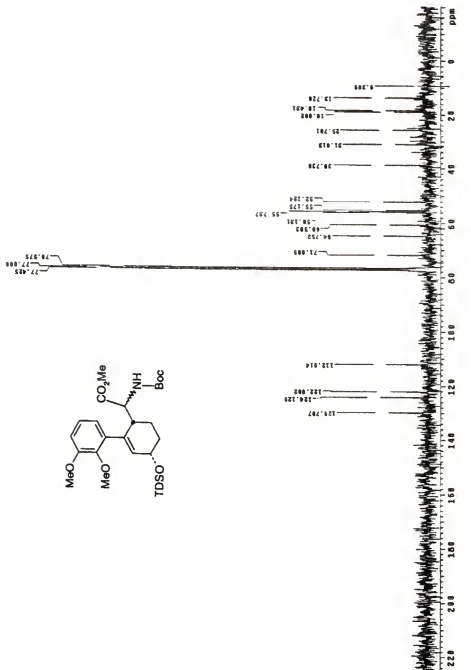
FREQUENCY 300.075 MHz
SPECTRAL WIDTH 4.5 kHz
ACQUISITION TIME 1.819 sec
RELAXATION DELAY 0.000 sec
PULSE WIDTH 45.0°
AMBIENT TEMPERATURE
NUMBER OF TRANSIENTS 1
DOUBLE PRECISION ACQUISITION
DATA PROCESSING
LINE BROADENING 1.0 Hz
FT SIZE 131072
TOTAL ACQUISITION TIME 1 minutes
MeO
COOMe
NH-Boc
TBSO
ppm

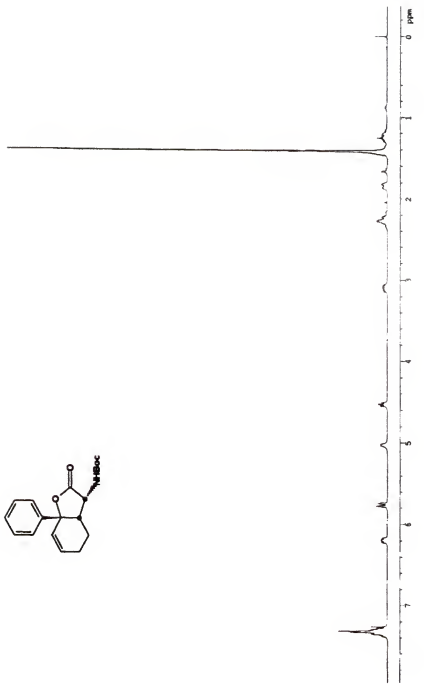
```
OBSERVE C13
  FREQUENCY 75.462 MHz
  SPECTRAL WIDTH 19761.7 Hz
  ACQUISITION TIME 0.800 sec
  RELAXATION DELAY 0.000 sec
  PULSE WIDTH 5.0 usec
  AMBIENT TEMPERATURE
  NO. REPETITIONS 1152
DECOUPLE H1
  LOW POWER 1023
  DECOUPLER CONTINUOUSLY ON
  WALTZ16 MODULATED
DOUBLE PRECISION ACQUISITION
DATA PROCESSING
  LINE BROADENING 3.5 Hz
  FT SIZE 32768
TOTAL ACQUISITION TIME IS minutes
```



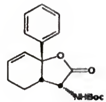


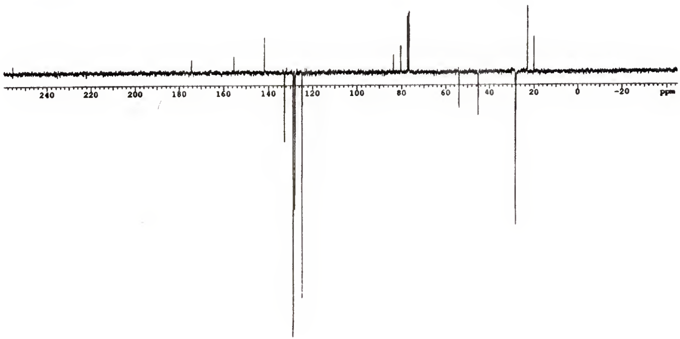

MeO
MeO
TDSO
CO2Me
NH
Boc
ppm

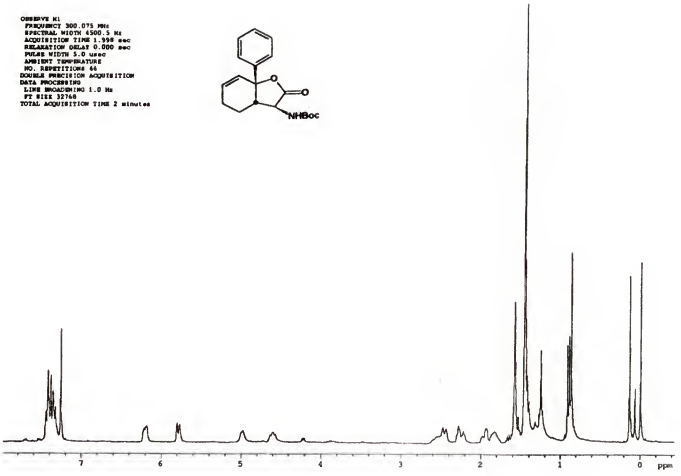

MeO
MeO
TDSO
CO2Me
NH
Boc
ppm


NHBoc
ppm
0
1
2
3
4
5
6
7

PULSE SEQUENCE apt
OBSERVE C13
FREQUENCY 75.430 MHz
SPECTRAL WIDTH 22988.5 Hz
ACQUISITION TIME 1.818 sec
RELAXATION DELAY 0.000 sec
PULSE WIDTH 8.7 usec
FIRST PULSE WIDTH 26.0 usec
AMBIENT TEMPERATURE
NO. REPETITIONS 640
DECOUPLE H1
HIGH POWER 44
DECOUPLER GATED ON DURING ACQUISITION
WALTZ16 MODULATED
DATA PROCESSING
LINE BROADENING 0.2 Hz
FT SIZE 262144
TOTAL ACQUISITION TIME 19 minutes

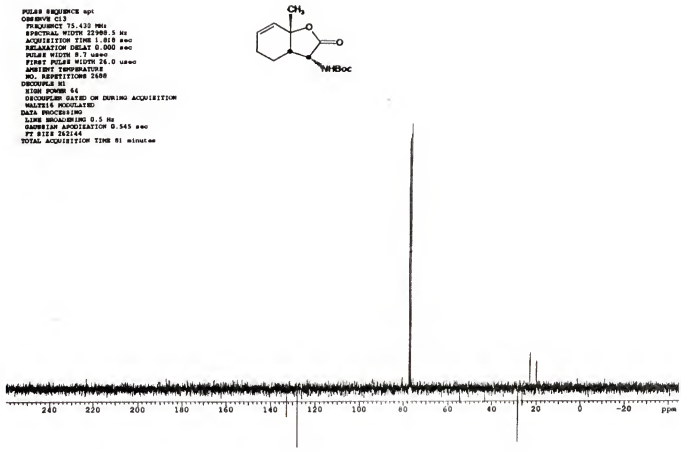


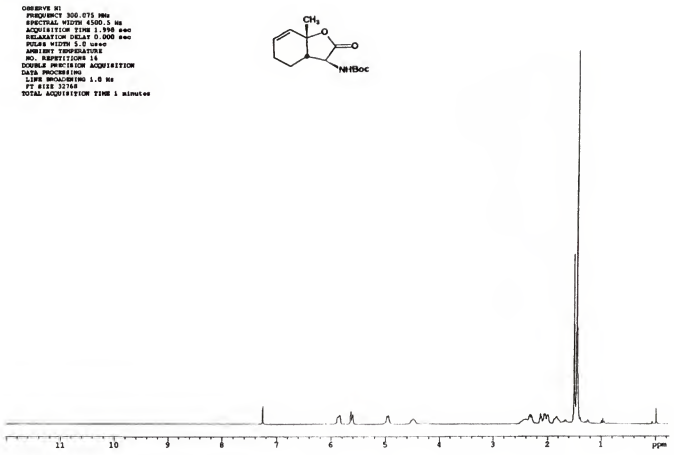



OBSERVE H1
FREQUENCY 300.075 MHz
SPECTRAL WIDTH 4500.5 Hz
ACQUISITION TIME 1.998 sec
RELAXATION DELAY 0.000 sec
PULSE WIDTH 5.0 usec
AMBIENT TEMPERATURE
NO. REPETITIONS 64
DOUBLE PRECISION ACQUISITION
DATA PROCESSING
LINE BROADENING 1.0 Hz
FT SIZE 32768
TOTAL ACQUISITION TIME 2 minutes
O
O
NHBoc
7
6
5
4
3
2
1
0
ppm

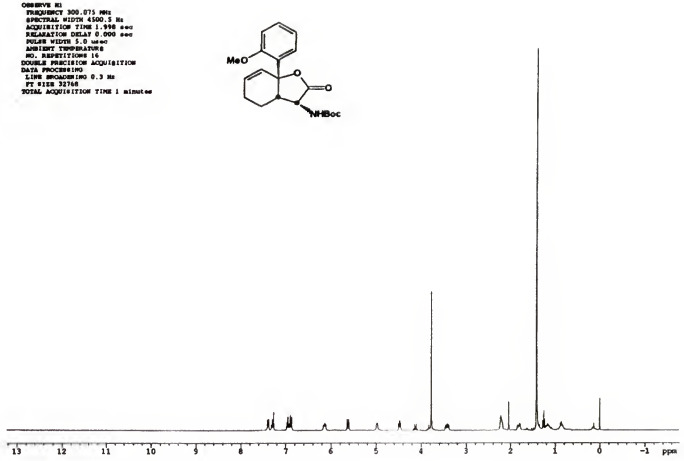
NHBoc
ppm

CH3
NHBoc
ppm

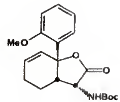

PULSE SEQUENCE apt
OBSERVE C13
FREQUENCY 75.430 MHz
SPECTRAL WIDTH 22988.5 Hz
ACQUISITION TIME 1.818 sec
RELAXATION DELAY 0.000 sec
PULSE WIDTH 8.7 usec
FIRST PULSE WIDTH 26.0 usec
AMBIENT TEMPERATURE
NO. REPETITIONS 2688
DECOUPLE H1
HIGH POWER 64
DECOUPLER GATED ON DURING ACQUISITION
WALTZ16 MODULATED
DATA PROCESSING
LINE BROADENING 0.5 Hz
GAUSSIAN APODIZATION 0.545 sec
FT SIZE 262144
TOTAL ACQUISITION TIME 81 minutes
CH3
O
O
NHBoc
240
220
200
180
160
140
120
100
80
60
40
20
0
-20
ppm

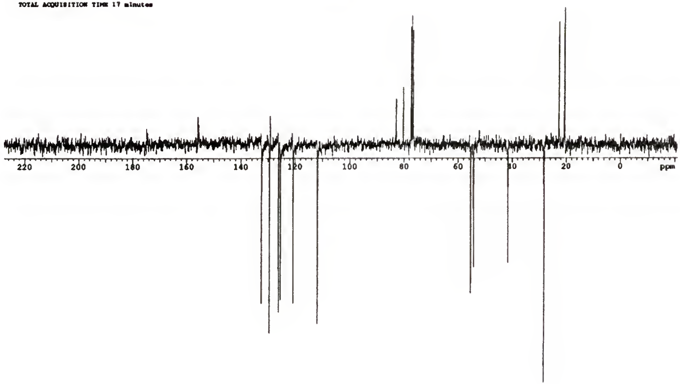

OBSERVE H1
FREQUENCY 300.075 MHz
SPECTRAL WIDTH 4500.5 Hz
ACQUISITION TIME 1.998 sec
RELAXATION DELAY 0.000 sec
PULSE WIDTH 5.0 usec
AMBIENT TEMPERATURE
NO. REPETITIONS 16
DOUBLE PRECISION ACQUISITION
DATA PROCESSING
LINE BROADENING 1.0 Hz
FT SIZE 32768
TOTAL ACQUISITION TIME 1 minutes
CH3
O
O
NHBoc
11
10
9
8
7
6
5
4
3
2
1
ppm

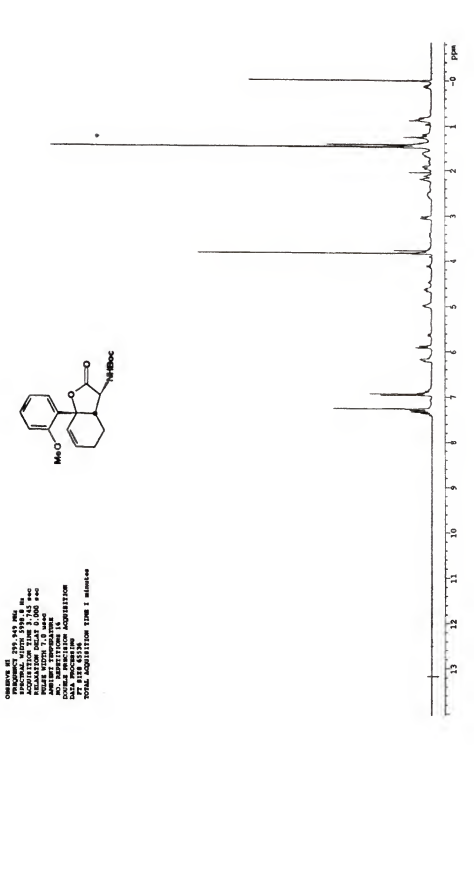
CH3
O
O
NHBoc
240
230
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
ppm


OBSERVE H1
FREQUENCY 300.075 MHz
SPECTRAL WIDTH 4500.5 Hz
ACQUISITION TIME 1.998 sec
RELAXATION DELAY 0.000 sec
PULSE WIDTH 5.0 usec
AMBIENT TEMPERATURE
NO. REPETITIONS 16
DOUBLE PRECISION ACQUISITION
DATA PROCESSING
LINE BROADENING 0.3 Hz
FT SIZE 32768
TOTAL ACQUISITION TIME 1 minutes
MeO
O
O
NHBoc
13
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
ppm

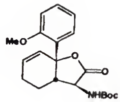
PULSE SEQUENCE apt
OBSERVE C13
FREQUENCY 75.462 MHz
SPECTRAL WIDTH 10761.7 Hz
ACQUISITION TIME 0.800 sec
RELAXATION DELAY 0.000 sec
PULSE WIDTH 5.0 usec
FIRST PULSE WIDTH 31.8 usec
AMBIENT TEMPERATURE
NO. REPETITIONS 1280
DECOUPLE H1
HIGH POWER 10
DECOUPLER GATED ON DURING ACQUISITION
WALTZ16 MODULATED
DOUBLE PRECISION ACQUISITION
DATA PROCESSING
LINE BROADENING 3.5 Hz
FT SIZE 32768
TOTAL ACQUISITION TIME 17 minutes

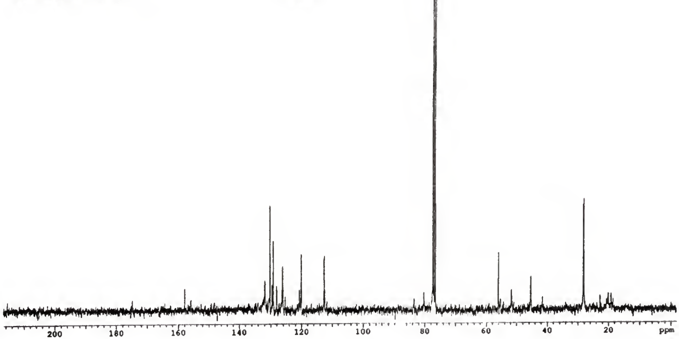


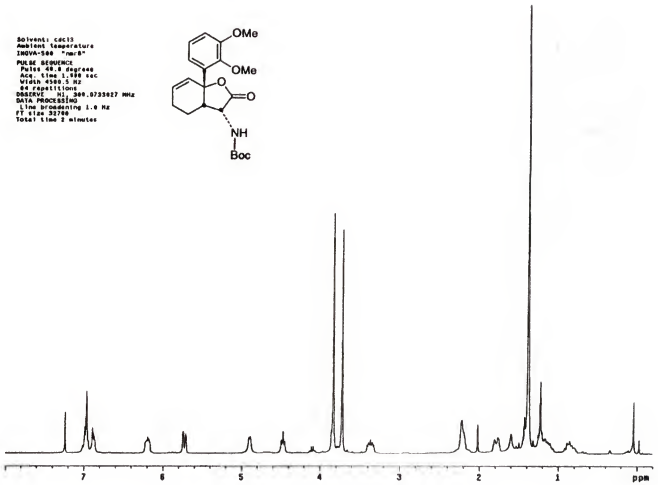


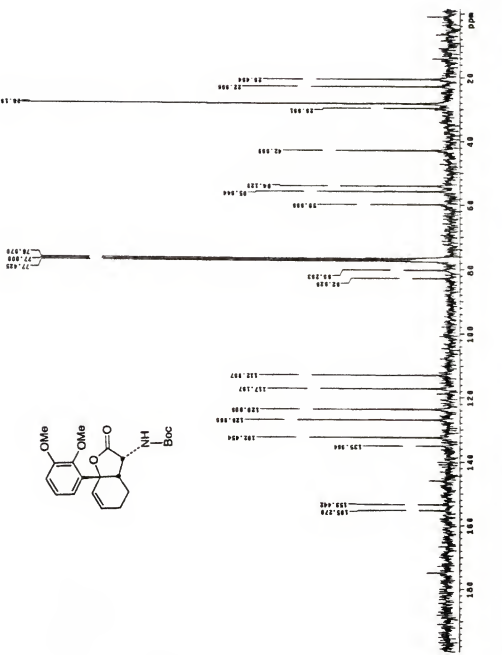

OBSERVE H1
FREQUENCY 399.945 MHz
SPECTRAL WIDTH 5000.0 Hz
ACQUISITION TIME 3.745 sec
RELAXATION DELAY 1.000 sec
PULSE WIDTH 7.0 usec
MODE: FOURIER TRANSFORM
NO. REPETITIONS 16
DATA ACQUISITION
DATA PROCESSING
FT SIZE 65536
TOTAL ACQUISITION TIME 1 minutes
MeO
NHBoc
ppm

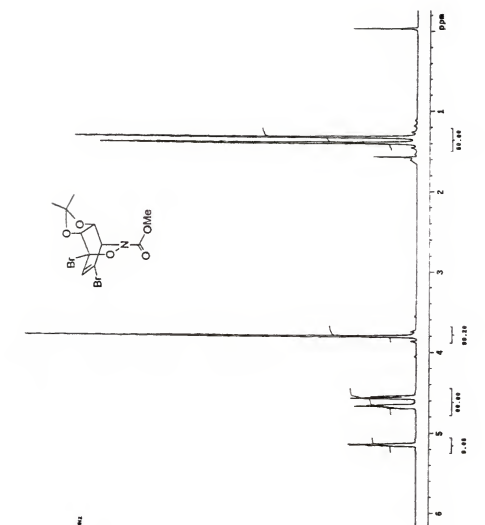
OBSERVE C13
FREQUENCY 75.430 MHz
SPECTRAL WIDTH 16501.7 Hz
ACQUISITION TIME 1.815 sec
RELAXATION DELAY 0.000 sec
PULSE WIDTH 8.7 usec
AMBIENT TEMPERATURE
NO. REPETITIONS 3520
DECOUPLE H1
HIGH POWER 64
DECOUPLER CONTINUOUSLY ON
WALTZ16 MODULATED
DATA PROCESSING
LINE BROADENING 2.0 Hz
FT SIZE 65536
TOTAL ACQUISITION TIME 106 minutes

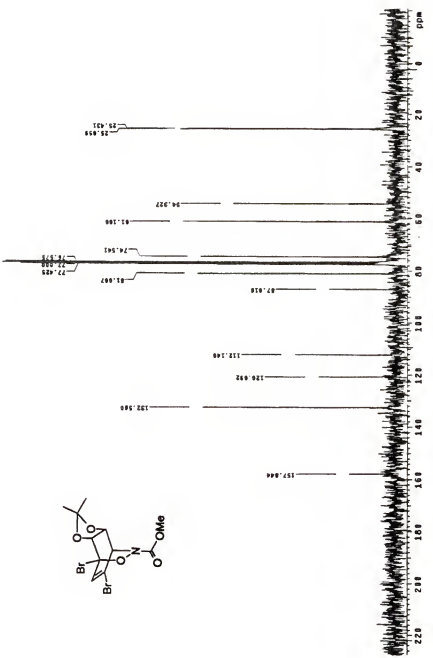


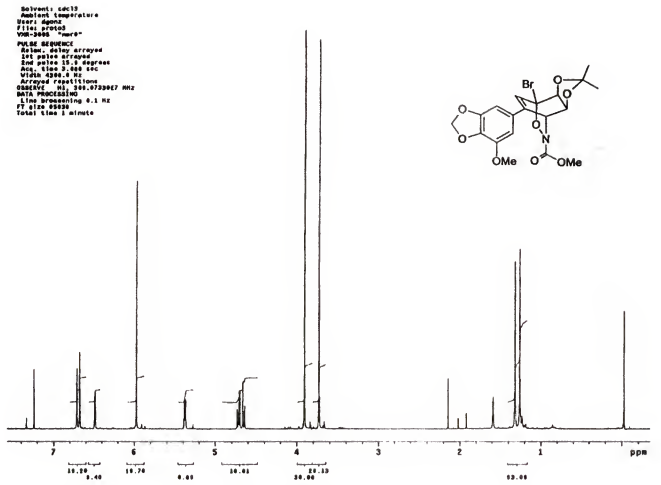


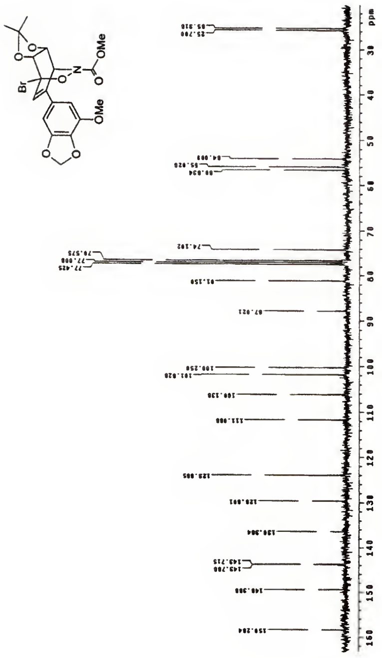

Solvent: cdcl3
Ambient temperature
INOVA-500 "nmr5"
PULSE SEQUENCE
Pulse 49.8 degrees
Acq. time 1.998 sec
Width 4500.5 Hz
No. repetitions 1
OBSERVE H1, 499.8733027 MHz
DATA PROCESSING
Line broadening 1.0 Hz
FT size 32768
Total time 2 minutes
OMe
OMe
O
O
NH
Boc
7
6
5
4
3
2
1
ppm

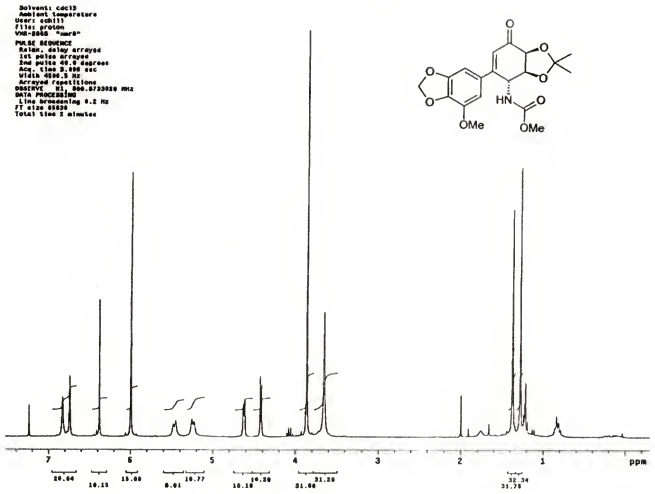

OMe
OMe
O
O
NH
Boc
ppm

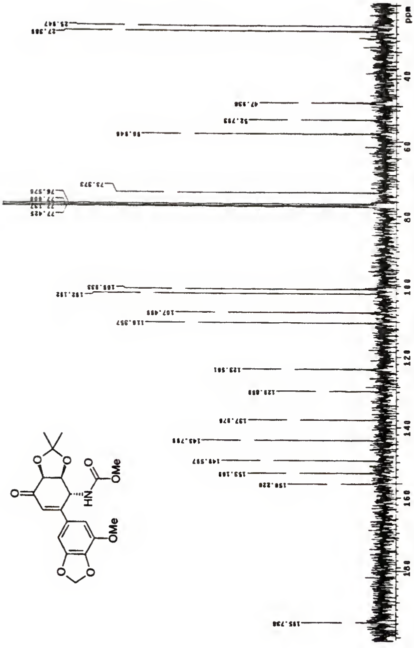

Br
Br
O
O
N
O
O
OMe
ppm

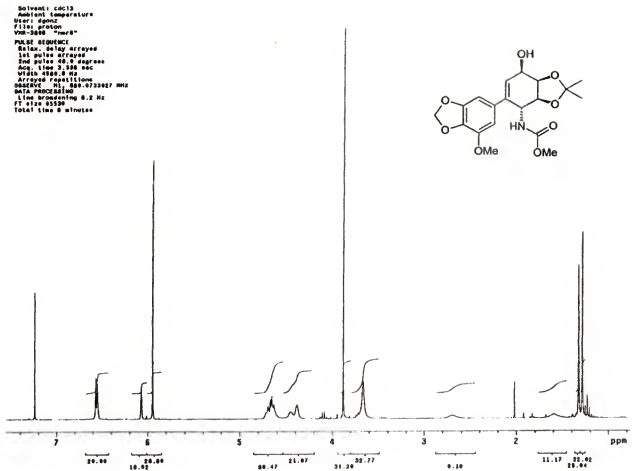

ppm
0
20
40
60
80
100
120
140
160
180
200
220
25.431
25.859
54.327
61.166
74.541
76.978
77.000
77.425
81.097
87.618
112.149
120.692
132.560
157.844
Br
Br
O
O
N
O
OMe

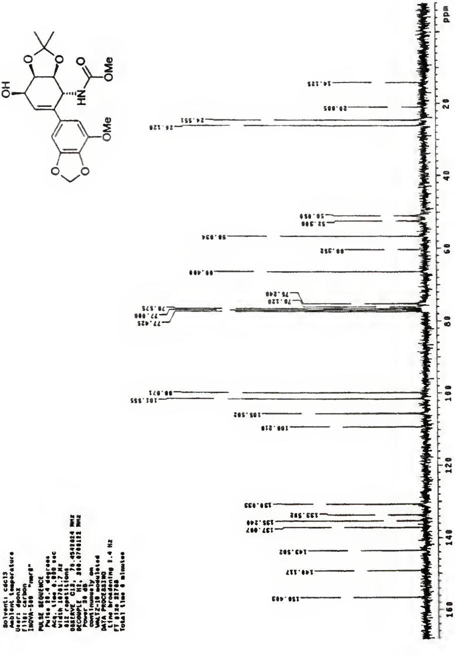

Solvent: cdcl3
Ambient temperature
User: dgoss
File: proto3
VNS-300S "nmr5"
PULSE SEQUENCE
Relax. delay arrayed
1st pulse arrayed
2nd pulse 19.1 degrees
Acq. time 3.986 sec
Width 4998.8 Hz
Arrayed repetitions
OBSERVE H1, 300.0720967 MHz
DATA PROCESSING
Line broadening 0.1 Hz
FT size 65536
Total time 1 minute
Br
O
O
O
O
O
N
O
OMe
O
OMe
7
6
5
4
3
2
1
ppm
10.00
9.40
10.70
4.69
10.01
20.15
20.00
23.06


Br
OMe
OMe
ppm

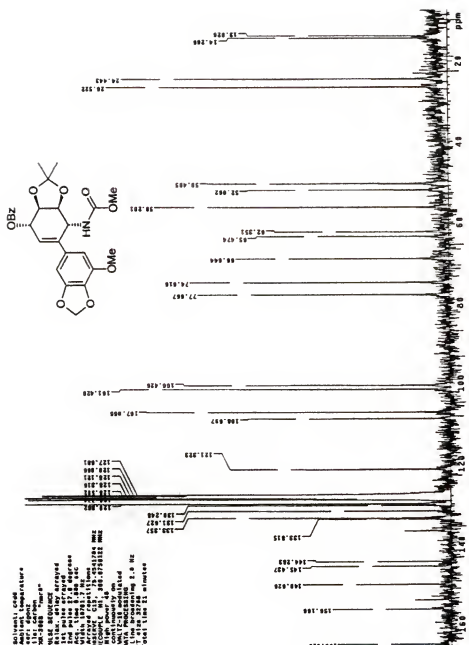

Solvent: cdcl3
Ambient temprature
User: achill
File: pr0ben
VNM-2868 "mara"
PULSE SEQUENCE
Relax. delay arrayed
1st pulse arrayed
2nd pulse 45.0 degrees
Acq. time 2.998 sec
Width 4696.5 Hz
Arrayed repetitions
OBSERVE H1, 866.5733920 MHz
DATA PROCESSING
Line broadening 0.2 Hz
FT size 65536
Total time 2 minutes
O
O
O
O
O
HN
O
OMe
OMe
7
6
5
4
3
2
1
ppm
20.04
10.15
10.00
8.01
10.77
10.10
10.20
31.00
31.20
32.34
31.79



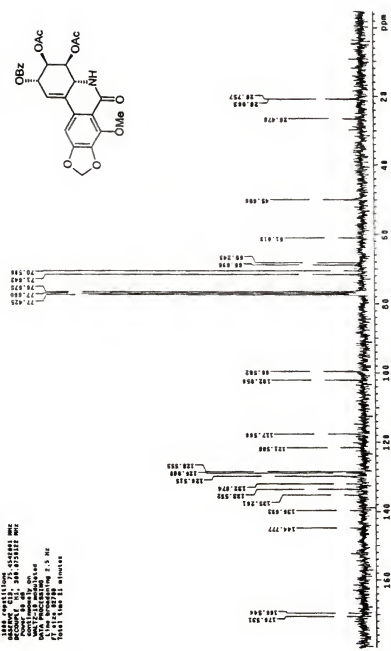

Solvent: cdcl3
Ambient temperature
User: dgonz
File: proton
VNM-300B "nmr8"
PULSE SEQUENCE
Relax. delay arrayed
1st pulse arrayed
2nd pulse 90.0 degrees
Acq. time 3.898 sec
Width 4500.0 Hz
Arrayed repetitions
SAMPLE CONSOLE 399.8733027 MHz
DATA PROCESSING
Line broadening 0.2 Hz
FT size 65536
Total time 8 minutes
OH
HN
OMe
OMe
ppm
29.00
20.00
10.05
80.47
21.07
31.30
32.77
0.10
11.17
22.92
25.94

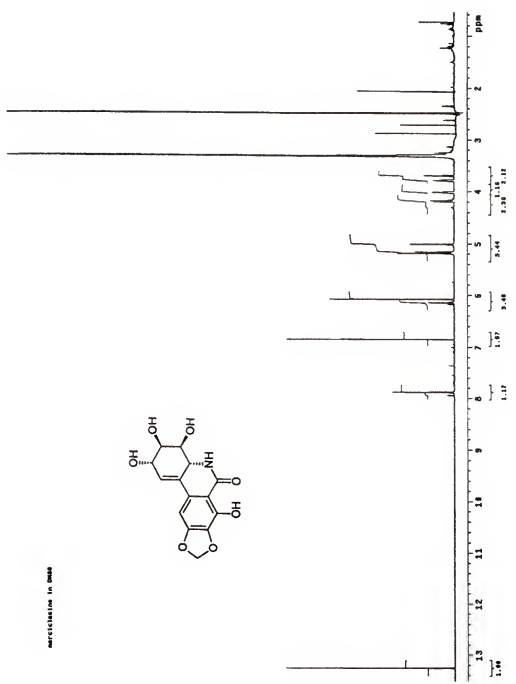


OBz
HN
O
OMe
OMe
O
O
O
O
ppm

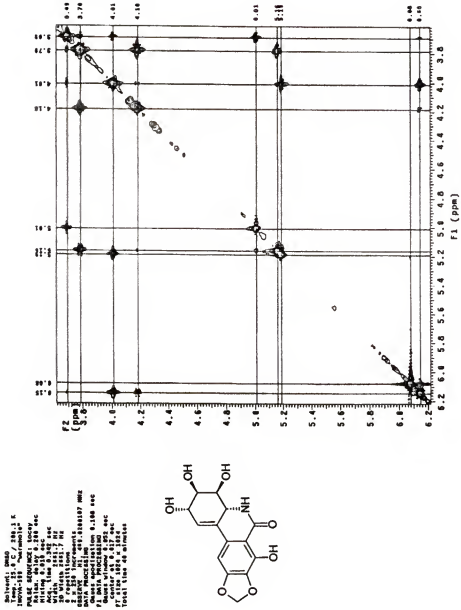

OBz
OMe
HN
OMe
O
O
O
O
O
ppm

OBz
OAc
OAc
NH
OMe
O
ppm


OBz
OAc
OAc
NH
OMe
O
ppm


OH
OH
NH
O
OH
O
O
ppm
1
2
3
4
5
6
7
8
9
10
11
12
13
1.00
1.17
1.97
3.40
2.44
1.16
2.10
2.18

OH
OH
NH
O
OH
O
O
ppm



BIOGRAPHICAL SKETCH

Carlos David Gonzalez was born in Montevideo, Uruguay, in 1965. He attended elementary and middle school at Instituto Crandon and undergraduate education at the School of Chemistry of the Uruguayan public university, Universidad de la República. He performed undergraduate research in the natural products laboratory of Professor Patrick Moyna, where he later worked as lab technician. In 1994, he completed his master's degree under Professor Eduardo Manta in the area of bioactive marine natural products. He was later accepted as a graduate student at the University of Florida, where he joined the group of Professor Tomas Hudlicky. His current interests of research involve the use of microbial biotransformations as tools for organic synthesis. The results of his research have been presented at several meetings and have resulted in eight publications.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Tomas Hudlicky, Chairman
Professor of Chemistry

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Merle A. Battiste
Professor of Chemistry

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Eric Enholm
Associate Professor of
Chemistry

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Ben Horenstein
Assistant Professor of
Chemistry

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

James F. Preston III
Professor of Microbiology and
Cell Science

This dissertation was submitted to the Graduate Faculty of the Department of Chemistry in the College of Liberal Arts and Sciences and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August 1999

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Dean, Graduate School